ELEVATED CO_2 IN CONTROLLED ATMOSPHERE STORAGE AND REGULATION OF MANGO RIPENING

By

RENAR JOÃO BENDER

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To my mother, Dulce Graebin Bender,
and to the memory of my father,
Eno Breno Bender.

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LIST OF ABBREVIATIONS

ACC	1-aminocyclopropane-1-carboxylic acid
ACP	anaerobic compensation point
ADH	alcohol dehydrogenase
ATP	adenosine triphosphate
CA	controlled atmosphere
CI	chilling injury
EL	electrolyte leakage
IB	internal breakdown
LDH	lactate dehydrogenase
MA	modified atmosphere
N	Newton
NAD^+	oxidized nicotinamide adenine dinucleotide
NADH	reduced nicotinamide adenine dinucleotide
NBD	norbornadiene
PDC	pyruvate decarboxylase
PFK	ATP-dependent phosphofructokinase
PFP	pyrophosphate dependent phosphofructokinase
RC	respiratory control
SDH	succinate dehydrogenase
TCA	tricarboxylic acid cycle

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Renar João Bender

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Mangoes (*Mangifera indica* L.) have grown considerably in economic importance in recent years. Starting out as a specialty product, mangoes have changed in status to a standard item in produce departments. Air shipment has given way to marine transport, resulting in harvesting and handling practices that have sacrificed quality. Implementing a tree ripe program for mangoes, resembling the trends in the deciduous fruit industry, probably constitutes the best alternative to improve the quality of the fruit on the market.

To avoid quality losses caused by mandatory quarantine treatments, insecticidal atmospheres have been tested for fruit fly disinfestation. It was concluded in those tests that mangoes have high tolerance to short term elevated CO_2 atmospheres. This is a characteristic of mango physiology that was not determined in the early CA research done with mangoes.

Based on this information, the applicability of longer term elevated CO_2 storage was evaluated. In these experiments, it became evident that mangoes can tolerate CO_2 atmospheres of up to 25% for 2 or more weeks at 12°C. Mangoes from higher CO_2 atmospheres were unable to resume ripening processes after transfer to air at 20°C.

Symptoms of damage were abnormal epidermal color, off-flavors and high ethanol production rates.

Evaluations of O_2 concentrations as low as 2% in the storage atmosphere indicated that reduction of O_2 concentrations did not induce higher respiratory activity, but at 2 or 3% O_2, depending on the cultivar, ethanol and acetaldehyde production were significantly enhanced. The reduction of the O_2 concentrations had an effect on hue angle similar to CO_2 during storage and after transfer to air, which may be attributed to reduced chlorophyll breakdown.

Reduction of the storage temperature to 8 or 5°C in order to further reduce ripening processes indicated that 5°C may be the lower limit as there was evidence of chilling injury, suggested by higher electrolyte leakage, even though no typical visual symptoms were manifested. The 25% CO_2 atmosphere caused injury to the mangoes at 5°C, indicated by scalding on the peel and also by significantly higher ACC concentrations in mesocarp tissue. These higher ACC concentrations were presumably a consequence of suppression of the activity of ACC oxidase by the 25% CO_2.

In general, throughout the experiments, CO_2 concentrations of 25% and above completely inhibited ethylene production and, at the same time, ethanol production increased substantially, though these elevated ethanol levels were not the cause of the suppression of ethylene production. The effect of CO_2 on ethylene biosynthesis may be ascribed more directly to a reversible inhibition of ACC oxidase. The effects of CO_2 on ACC synthase activity does not seem to be as evident in mangoes as in other fruits.

Aroma volatiles were reduced following 25% CO_2 treatment, while 10% CO_2, low O_2 atmospheres and storage temperature did not seem to significantly influence the production of terpene hydrocarbons, the compounds that have been suggested to be characteristic of Florida-type mangoes.

CHAPTER 1
INTRODUCTION

Mangoes (*Mangifera indica* L.) have grown considerably in importance on the international market, not only in terms of volume but also in terms of value traded (Subrahmanyam, 1990), and are listed second to bananas among tropical fruits (Gourgue *et al.*, 1992). The market for tropical fruits in industrialized countries has expanded over the past few years because of increased demand for exotic tastes and flavors. Overall, consumers have also become familiarized with distinct flavors that had previously been unique to ethnic groups.

The international market for mangoes has been increasing steadily since the beginning of the 1980s. In the U.S., the leading mango importer, the trade of mangoes has been growing rapidly (Subrahmanyam, 1990). Haines (1991) reported that import volumes increased about 10% yearly over part of the last two decades. According to Proctor (1990), mangoes and avocados are the fruits with the greatest potential for increased commercialization from among a group of moderately and well established species. For mangoes to fulfill their economic potential and attain higher ranks of popularity will depend, in part, on improved transportation and storage (Tucker and Seymour, 1991).

Although higher ranks in popularity may be attained by utilizing new marketing strategies, consumer acceptance is more responsive to the quality attributes of the product itself and some consumers may be willing to pay premium prices for top quality product

(Bruhn *et al.*, 1991). Ripe mangoes are rich in pro-vitamin A, have moderate concentrations of vitamin C and small amounts of the vitamin B group. Mangoes are also rich in pectic substances and could represent a fiber source with a positive influence on human metabolism (Gourgue *et al.*, 1992). The dietary properties, though, can be overshadowed by lack of visual quality, which is the factor, together with increased availability at the retail level, that most significantly influences demand and market growth (Proctor, 1990).

Mangoes do not store well for periods longer than 2 to 3 weeks. Air shipments have been unable to handle the increased tonnage of recent years and therefore marine shipment with low temperature storage has been the sole alternative to move the larger fruit volumes (Medlicott *et al.*, 1990). The problem with marine shipments is the time a vessel takes to reach consumer markets. Depending on the departing port, it may take up to 3 weeks to reach markets in Europe and the USA (Medlicott *et al.*, 1990).

Fruit softening in mango occurs very rapidly and is one of the causes of quality deterioration during postharvest handling. Excessive softening renders fruit more susceptible to impact and compression bruises and establishment of postharvest pathogens (Mitcham and McDonald, 1992). In order to avoid the excessive softening of fully mature mangoes, the fruit are harvested at earlier stages, resulting in partially firmer fruit, but with inevitable effects on the final quality. Consequently, there is a need for the development of storage conditions to delay softening and ripening, thus allowing supply of distant markets with higher quality fruit.

In the 1960s, researchers, envisioning the market potential of mangoes, started to investigate more intensively the applicability of controlled atmosphere (CA) storage to shelf life extension, which would help to consolidate the fruit on the international market. The various combinations of atmospheres (low oxygen [O_2], high carbon dioxide[CO_2]) and temperatures tested, however, did not clearly point to advantages of applying CA storage to mangoes. Determining a single, optimum combination of O_2 and CO_2 has been more difficult because of the multitude of cultivars from genetically different groups.

The mandatory fruit fly disinfestation treatments required by importing countries pose another threat to mango postharvest quality. Because of heat treatment injuries to the fruit, insecticidal atmospheres have been examined as alternatives. These experiments have led to the conclusion that mangoes are more tolerant to short term elevated CO_2 atmospheres (Yahia *et al.*, 1989) than most other tropical fruit species, which is an aspect of mango physiology that did not become apparent in the early CA work.

Considering that short exposures to extreme CO_2 concentrations did not cause injuries to mango fruit and that longer storage under moderately high CO_2 atmospheres did not lead to conclusive results, the first objective in the present work was to verify the applicability of extreme CO_2 concentrations for storage times equivalent to the duration of marine export shipping and to determine the levels at which damage to mango fruit will occur. Long term storage was considered as the time for transport from the production areas to receiving ports overseas, estimated to be a maximum of 3 weeks.

Although the general recommendation for refrigerated storage of mangoes is storage between 12 and 13°C (Kader, 1993), a wide range of temperatures has been suggested in the literature. Based on evidence that a modification of the atmosphere alleviated the occurrence of chilling injury symptoms in mangoes (Chaplin *et al.*, 1991; Tucker and Seymour, 1991) and that mangoes in which ripening had been initiated were less sensitive to chilling temperatures (Chaplin *et al.*, 1991), a second objective was established to determine the effectiveness of the combination of CA treatments with reduced storage temperature to prolong shelf life of tree ripe mangoes.

Because of the many possible sites of CO_2 in reducing metabolic activity during CA storage and, moreover, because mangoes have shown less sensitivity to elevated CO_2 concentrations than many other crops, subjecting intact fruit or mango tissue to elevated CO_2 concentrations might help to elucidate further the mode by which CO_2 limits metabolism and thus prolongs shelf life.

One of the first models proposed to explain how CO_2 could retard ripening processes during storage suggested inhibition of ethylene action (Kidd and West, 1945; Burg and Burg, 1967). The continuous attention to the ethylene biosynthetic pathway to further understand its regulatory mechanism and responses to external factors, such as elevated CO_2 atmospheres, led to some controversial results, which are quite highly species-dependent (Mathooko, 1996). Yang and Hoffman (1984) determined that 1-aminocyclopropane-1-carboxylic acid (ACC) synthase is the regulatory step in the pathway and all the work thereafter corroborated the key role of the enzyme in ethylene biosynthesis (Kende, 1993). However, evidence has accumulated that ACC oxidase (formerly known as ethylene forming enzyme [EFE]), the last step in the pathway, is differentially affected by CO_2 (Mathooko, 1996). In general, elevated CO_2 atmospheres have shown inhibitory effects on ethylene synthesis in fruit tissues, though, for example Bufler (1986) and Rothan and Nicolas (1994) have reported that short term exposures of apple and kiwifruit tissue to elevated CO_2 concentrations enhanced ACC oxidase activity. Therefore, the third objective was to investigate the overall effects of elevated CO_2

atmospheres on mango ethylene production and, more specifically, determine the effects of these atmospheres on the *in vivo* and *in vitro* activity of ACC oxidase.

Tree ripe or physiologically mature mangoes allowed to ripen at ambient conditions fully express the flavor characteristics of the cultivar although with significantly reduced shelf life compared to fruit harvested at the mature green stage. If ripening proceeds at temperatures above 28°C, the fruit may develop off-flavors, while, on the other hand, fruit held in refrigerated storage (below 15°C) have inferior flavor characteristics compared to fruit ripened at temperatures above 15°C (Lakshminarayana, 1980).

There is little information with regard to the response of the aroma components of mangoes to CA and/or modified atmosphere (MA). The majority of the literature available on mango volatiles deals with the characterization of profiles and with evaluations of the different mango cultivars of economic importance. In addition, there have been many informal and fewer formal evaluations of mango flavor over the course of the years. However, based on the knowledge that CA and MA, more specifically extreme concentrations of either CO_2 or O_2, significantly suppress aroma evolution in species like apples, aroma volatiles of mangoes stored under elevated CO_2 atmospheres were examined. Finally, as part of this last objective, differences in aroma profiles between mature green and tree ripe mangoes and mangoes stored at the actual recommended temperature, 12°C, and at two temperatures below this recommendation, 5 and 8°C, were also determined.

CHAPTER 2
LITERATURE REVIEW

Controlled and Modified Atmospheres

The use of storage methods that modify the atmosphere surrounding agricultural commodities dates back to ancient times. Placing dried grains into sealed clay pots or underground pits was practiced by pre-Neolithic societies of the Middle East about 10,000 years ago. Some of these underground and above the ground structures are still in use by farmers around the world. The use of large pits and silos, which had turned into major procedures for long-term storage during the Roman era, may be considered precursors of today's MA storage (Kays, 1991).

In both MA and CA, the atmosphere surrounding the product is altered. O_2 concentrations are changed from the atmospheric 20.8% to lower levels depending on commodity tolerance limits, while CO_2 concentrations are changed from the atmospheric 0.03% to higher levels depending, again, on the tolerance of the commodity to the elevated concentration. The distinction between MA and CA is the way the atmospheres are established and the level of control and adjustment of those assigned concentrations. Under MA, the commodity itself, over time, alters the surrounding atmosphere by the ongoing consumption of O_2 and production of CO_2 by the metabolic processes in an enclosed environment with basically very limited control over the adjustment. CA is a direct alteration of the storage atmosphere by means other than the metabolism of the stored product and followed by continuous monitoring and adjustment of the established concentrations.

The physiological basis for the beneficial effects of MA and CA are basically related to reduced metabolism of the stored commodity under altered atmospheric storage conditions (Smock, 1979; Kader, 1986). Generally the reduction of either O_2 levels or the elevation of CO_2 results in a proportional reduction in respiratory activity (Kader, 1986). Under low O_2 atmospheres, both the changes in O_2 uptake and CO_2 production remain proportional with no alteration of the respiratory quotient (RQ), *i.e.*, CO_2 production over O_2 consumption while, under elevated CO_2 atmospheres, the CO_2 production is affected more than O_2 uptake, thus leading to differences in RQ under different CO_2 levels (Knee, 1973). These differences in CO_2 production may derive from shifts in the substrate used for respiration under the different atmospheres and/or combined with the occurrence of some fermentation (Joles *et al.*, 1994; Chervin *et al.*, 1996) or from incomplete oxidation, *i.e.*, oxidation inhibited at some specific step (Phan *et al.*, 1975).

Reduced respiratory activity in low O_2 at levels typically used for MA or CA does not seem to be derived from suppressed basal metabolism via terminal cytochrome oxidases, but from external oxidases that have much higher K*m* values for O_2 (Burton, 1974). Low O_2 also exerts its action by suppressing *de novo* synthesis of proteins in ripening fruits and also induces synthesis of new proteins regardless of the developmental stage of the fruit (Kanellis *et al.*, 1989b). The authors observed that there were changes in polypeptide profiles after subjecting avocados to low O_2 atmospheres. Moreover, since O_2 is required for ethylene biosynthesis (Yang, 1980) and action (Kanellis *et al.*, 1989a), it has also been considered as a possible mechanism by which reduced O_2 delays fruit ripening.

The primary sites of CO_2 action in CA/MA are on enzymes of the tricarboxylic acid (TCA) cycle, mainly succinate dehydrogenase (SDH) (Knee, 1973; Monning, 1983), while the effects on other enzymes of the TCA cycle seem to be more negligible. Activity of glycolytic enzymes has also been shown to be reduced under elevated CO_2. Kerbel *et al.* (1988) determined that 10% CO_2 in the storage atmosphere significantly reduced the activity of both the adenosine tri-phosphate (ATP) and pyrophosphate (PPi)-dependent phosphofructokinases (PFK and PFP, respectively) in pear fruits. These authors also verified that 10% CO_2 plus air for 6 days significantly suppressed ethylene production. In fact, it has been suggested that CO_2 exerts its effects on metabolism via reducing ethylene action (Burg and Burg, 1967).

Research in CA storage began in the 1920s with the work of Franklin Kidd and Cyril West in England (Dilley, 1978; Laties, 1995). Even though the first commercial CA facility was built shortly after the first publication of the recommendations of Kidd and West (Kays, 1991), the use of the technology only started to expand in the last 20 or 30 years. Apples and pears were the first major crops to benefit from CA storage, which was initially conceived to extend the storage period and in that way widen the marketing season with improved quality retention compared to regular refrigerated storage.

Because of the benefits of CA storage to apples and pears, the technology has been tested for species other than those temperate zone crops. Crops potentially amenable to CA or MA storage are those with significantly shorter shelf life periods and where chilling injury imposes limits to the use of reduction of storage temperature as the sole method for extending storage life.

The effort to limit ripening processes in temperate species during CA storage and, consequently, extend storage life consolidated harvests at preclimacteric stages. Though the ideal condition would be harvest at a maturity to allow full development of quality after storage, the procedure has sacrificed flavor (Dilley *et al.*, 1989). For example, Jobling and McGlasson (1995) observed reduced flavor after storage of an early season apple cultivar harvested at the preclimacteric stage compared to the harvest at a more advanced maturity. However, the preclimacteric apples were significantly firmer than late-harvested fruit or non-CA-stored apples. Flesh firmness is a very important parameter, not only in apple storage (Lau *et al.*, 1983; Weichmann, 1986), but also in mangoes (Mitcham and McDonald, 1992). Medlicott *et al.* (1988) and Seymour *et al.* (1990) observed that mangoes harvested at the mature green stage did undergo ripening processes as did tree ripe fruit, though more slowly, when stored at room temperature for 7 days or at 12°C for 21 days. These observations have only confirmed the practice of trade-off of quality characteristics in order to gain storage life.

Controlled Atmosphere Storage of Mangoes

Although Spalding and Reeder (1974) and Jordan and Smith (1993) have stated that little research had been done looking at the effectiveness of CA storage for tropical fruits, research examining the applicability of MA and CA for mangoes started very early. Probably the first report on MA storage of mangoes was published by Banerjee, Karmarkar and Row in 1934 (cited by Hatton and Reeder, 1965), in which the authors showed that over 15% CO_2 atmospheres caused abnormal color development but had no effects on flavor. In 1941, Karmarkar and Joshi concluded from their work with mature green 'Alphonso' mangoes that there was no effect on respiratory activity of CO_2 concentrations up to 11% for a short exposure period (about 10 hours) at 20°C. The O_2 levels used in this experiment were reduced concomitantly to the increase in CO_2, and the resulting reduction from 21% O_2 to 10% O_2 did not seem to have any inhibiting action on respiration rates. The authors did find indication of fermentation after 24 hours when CO_2 levels reached 20% and O_2 levels were about 7%.

Kapur *et al.* (1962), also working with mature green 'Alphonso' mangoes, considered the final quality of fruits stored under a 7.5% CO_2 atmosphere at 8-10°C for 35 days to be satisfactory, and the fruits ripened normally after transfer to air. Working with another cultivar, 'Raspuri', under the same conditions but for as long as 49 days at temperatures ranging from 5.5 to 7.2°C, there again was no quality or ripening impairment, with fruits ripening satisfactorily within 3 days in air. Mangoes of the control treatments for both cultivars at the end of storage period had less ascorbic acid, lower firmness and titratable acidity, and higher weight loss and wastage compared to CO_2-treated fruit.

Even though a 5% O_2 plus 5% CO_2 atmosphere proved to be the best combination for quality maintenance of 'Keitt' mangoes stored for 20 days at 12.8°C, Hatton and Reeder (1965) concluded that there did not seem to be enough advantage to warrant practical use of CA storage. The authors did mention, however, that fruit from the different atmosphere combinations were firmer than air-stored fruit and that these fruit also had significantly less weight loss. The highest CO_2 used in Hatton and Reeder's study was 9%, which after 30 days or more of storage produced unpalatable fruit. The O_2 concentration for this treatment was 4%. Off-flavors were detected as well in fruits stored in atmospheres with 1% O_2 plus 5% or 8% CO_2.

Increased alcohol and aldehyde concentrations were also found by Lakshminarayana and Subrahmanyam (1970) in ‘Alphonso’ mangoes stored under elevated CO_2 atmospheres at 12°C for 21 days, which was considered to be a consequence of enhanced anaerobic metabolism resulting in lower taste characteristics. The authors also suggested that CO_2 had a more controlling effect on anaerobiosis than O_2 and that concentrations initially set at 5%, 10% or 15% CO_2 inhibited carotenoid synthesis and, furthermore, resulted in lower visual color ratings of the fruit flesh.

Jordan and Smith (1993) stored mature green ‘Kensington’ mangoes for 4 and 6 weeks under a range of combinations from 0.5 to 2.2% O_2 plus 0 to 10% CO_2 at 10°C and found that only fruit from the 0.5% O_2 atmosphere had unacceptable taste. All the other combinations had similar rankings in eating quality. The authors also concluded that flesh firmness was affected only by O_2 concentrations. The lower the O_2 level, the firmer the mangoes.

O'Hare and Prasad (1993) concluded from their work with ‘Kensington’ mangoes that CO_2 concentrations over 10% at 7°C lead to high levels of ethanol, causing an off-odor and lower ratings of pulp color. CO_2 injury was identified after 3 weeks or more of storage as a change to an olive-khaki pattern in epidermal color, which the authors suggested was derived from oxidation of cellular or organelle-bound compounds leaking from injured cells due to changes in membrane fluidity. McLauchlan and Barker (1994) determined that color development of tree ripe ‘Kensington’ mangoes was only affected by CO_2 concentrations below 4%. There was no additional effect on color development at CO_2 levels between 4% and 10%, but the mangoes stored at those CO_2 levels for 33 days at 13°C were rated by a taste panel to have the best eating quality compared to control fruit and low O_2 atmospheres.

Noomhorn and Tiasuwan (1995) determined significant peel color differences between control and elevated CO_2 treatments of 'Rad' mangoes as measured by comparison to color charts after 25 days of storage at 13°C. Even though the ripening processes resumed after transfer to air, the sensory evaluation distinguished between 6% or 8% CO_2 and 4% CO_2. Mangoes from the higher CO_2 concentrations had a more pale yellow flesh color and some internal browning and off-flavors, which were presumed to be due to CO_2 injury. Based on these results, the authors recommended that mangoes be stored in an atmosphere of 6% O_2 plus 4% CO_2.

In contrast to the results mentioned above, Yahia *et al.* (1989), testing alternatives to current insect quarantine treatments, found that mangoes are very tolerant to elevated CO_2 atmospheres. When subjecting 'Keitt' mangoes to 50% CO_2 in combination with 2% O_2 for up to 5 days at 20°C, the authors observed that there was a delay in ripening processes. The high CO_2 treatment reduced fruit softening for up to 7 days after transfer to air compared to mangoes stored continuously in air. Even though there was a high CO_2 residual determined in the mangoes after transfer, no injury symptoms developed and no off-flavors were detected by sensory evaluation after one week in air at 20°C. Dominguez *et al.* (1993) came to the same conclusion that mangoes are tolerant of short term treatment, less than 3 days, with elevated concentrations of CO_2.

Low Temperature Storage and Chilling Injury of Mangoes

CA and MA have been examined in terms of their effects on specific sites of metabolism, but temperature, with a broader action upon metabolism, may also elicit a more definite response by tissues. The most evident of these responses is the occurrence of chilling injury. Chilling injury in mangoes has been reported to occur under a range of temperatures and also to be dependent on cultivar and ripeness stage (Chaplin *et al.*, 1986; Thomas and Joshi, 1988; Medlicott *et al.*, 1990; O'Hare and Prasad, 1993). The optimum temperature recommended for mature green mango storage is between 12 and 13°C (Medlicott *et al.*, 1987; Kader, 1993) with a possible range varying from 10 to 15°C depending on the cultivar and maturity or ripeness stage.

Chilling injury symptoms in mangoes are described as inhibition of ripening, browning, pitting and sunken lesions on the skin, pale yellow flesh color, low pH and increased levels of titratable acidity in the fruit flesh (Chaplin *et al.*, 1991; O'Hare and Prasad, 1993) and decreased resistance to fungal diseases (Thomas and Oke, 1983). Chhatpar *et al.* (1971) determined that chill-injured mangoes had less starch breakdown and ascorbic acid accumulation and also significantly lower sucrose levels compared to non-chilled mangoes.

Medlicott *et al.* (1990) mentioned the occurrence of a 'corky layer' about 5 to 7 mm underneath the peel as also being a manifestation of chilling injury. This 'corky layer' was observed in 'Tommy Atkins' mangoes harvested early in the season at an immature stage and stored for 21 days at 8 or 10°C. Half mature and mature mangoes also harvested early in the season and stored at 8°C also developed chilling injury. On the other hand, fully mature fruit stored at 12°C did not show any injury, but continued to ripen while in storage, significantly reducing the shelf life and marketing period.

Chaplin *et al.* (1991) did not observe chilling injury in mature green 'Kensington' mango fruits stored at 10°C for 3 weeks. Fruit stored at 1 and 5°C did, however, develop typical chilling injury symptoms. Upon transfer to a ripening room at 20°C, the severity of the injury increased. Severely injured fruit failed to ripen, while fruit with slight chilling injury had lower pH and, concomitantly, higher titratable acidity values in comparison to fruits kept at 20 and 15°C.

Thomas and Oke (1983) did not find any negative effects on the eating-quality of ripe 'Alphonso' mangoes that had been stored for 30 days at 10°C. However, under these conditions, 12% of the fruits showed skin bronzing and pitting. Storing the mangoes at 7 or 4°C only decreased the number of days required for symptom appearance after transfer for ripening at 27 to 32°C. Pre-conditioning preclimacteric fruit for 2 days with the temperature reduced either gradually or directly to 10°C before storage at 7 or 4°C, significantly increased the time required for symptom development. The authors hypothesized that a phase transition of cell membranes induced by cold adaptation could be the mechanism involved in reducing damage from low temperatures. Working with the same cultivar, Thomas and Joshi (1988) verified that fruit that had been grown and ripened at temperatures ranging from 27 to 34°C and were subsequently stored at 5, 10 or 15°C developed chilling injury symptoms within 2 to 4 days. Pre-conditioning these fruit for 3 days by holding the first day at 20°C, the second at 15°C, and finally at 10°C, with subsequent storage at 5 or 10 °C, only delayed the initial appearance of chilling injury symptoms.

The discrepancies in the effectiveness of the pre-conditioning treatments between the two previous reports may be attributed to the temperature difference and duration of the treatment having a significant effect on membrane compositional changes. Thomas and Joshi (1988) preconditioned the mangoes for 1 day at 20, 15 or 10 °C while Thomas and Oke (1983) used 2 days at 10°C.

Veloz *et al.* (1977) stored mature green 'Kent' mangoes at 8, 10 or 13°C for up to 22 days and reported that chilling injury occurred in all treatments. The incidence of chilling injury increased proportionately to the decrease in the storage temperature and to the increase in storage time. The mangoes stored at 8°C and then transferred to air at 25°C for 6 days failed to properly resume ripening processes, resulting in poor fruit quality as indicated by organoleptic evaluation. The authors considered that 13°C could probably be the critical temperature below which chilling injury occurs.

High Temperature Treatments and Heat Injury of Mangoes

Visual damage or loss of flavor are often caused by the hot water treatment (46.1°C [115°F] for 60 to 90 minutes) required by quarantine restrictions for mango shipments to mainland U.S. and to Japan and likewise required for mango shipments departing from Florida to the southwestern states of the U.S. (Sharp, 1992). Heat disinfestation methods, including hot water or vapor-heat, have been developed as alternatives to chemical treatments for disinfestation of mangoes of eggs and larvae of various species of fruit flies endemic to different growing areas (Jacobi and Wong, 1992).

Excessive shriveling, translucence, brown spots of the epidermis, and formation of cavities in the pulp (Sangchote, 1989; Joyce and Shorter, 1994) are some of the visual symptoms of heat treatment injury. Jacobi and Gowanlock (1995) found that parenchyma cells in the mesocarp tissue of heat-treated mango had thickened and convoluted cell walls. The authors concluded that the symptoms resembled those of chilling injury in peaches described by Luza *et al.* (1992) as leatheriness. Jacobi and Wong (1992) found high concentrations of starch granules in heat-injured mango tissues and reasoned that it was possibly due to enzyme inactivation by heat. Joyce *et al.* (1993) verified by magnetic resonance imaging that heat-injured mesocarp areas of mangoes were more starchy and showed up in the images as dark spots. Esguerra *et al.* (1990) attributed internal breakdown (IB), a physiological disorder of mango fruits induced by vapor heat treatments, to O_2 depletion in the innermost layers of mesocarp tissue, because fruits with IB also had off-flavors indicative of anaerobic metabolism.

Effects of Extreme Atmospheres on Mango Fruit Metabolism

Because of the negative effects of heat treatments on mango fruit quality and because the chemicals employed as fumigants for insect disinfestation have already been banned or face banning as a consequence of mutagenic and carcinogenic implications (Calvin *et al.*, 1984), attempts have been made to employ extreme O_2 and/or CO_2 atmospheres as insect quarantine treatments.

Reduced O_2 or elevated CO_2 storage atmospheres have been successfully employed for insect control in non-perishable commodities such as grains (Bell, 1984) and dried fruits (Soderstrom and Brandl, 1984). Many variables may affect the effectiveness of an insecticidal atmosphere and, so far, the technique is not employed commercially for fruits from the tropics, despite the potential for CA quarantine application as an alternative to the more costly heat treatment procedure.

Short term extreme CO_2 atmospheres applied to several commodities to minimize physiological disorders or to slow down fruit softening have proven to be beneficial. Wang and Mellenthin (1975) concluded that treating 'd'Anjou' pears with 12% CO_2 for up to 4 weeks resulted in the best quality ratings after 8 months of storage at -1°C. That concentration is 2 to 4 times the recommended level of CO_2 for long term pear storage (Claypool, 1973; Meheriuk, 1993). Pears from this treatment had higher malic acid content and did not show any injury symptoms. The authors also indicated that, although CO_2 concentrations of 26 and 42% caused injury to the peel, those treatments did not prevent the fruit from undergoing ripening processes and, while there were only slight differences in the ethylene climacteric, there were no differences in the respiration rates.

Lange (1988) reported a delay in softening of 'McIntosh' apples during air storage at 2.5°C when the fruit were pre-treated for 12 hours with 50% CO_2. Subjecting the apples to CO_2 concentrations varying from 10 to 30% for 7 to 21 days caused severe CO_2 peel injury, which did not occur under the short term treatment.

Sitton and Patterson (1992) found that atmospheres enriched with up to 12% CO_2 resulted in less incidence of decay in 'Golden Delicious', 'McIntosh' and 'Delicious' apples than at a 0.5% CO_2 atmosphere after a 61 day exposure at 0°C. No significant effects on organoleptic parameters were detected in apples from this high CO_2 treatment, which is 3 to 4 times over the concentration recommended for longer storage times (Meheriuk, 1993).

Ke *et al.* (1990) exposed 'Bartlett' pears to 20, 50 or 80% CO_2 for up to 10 days at 5 or 10°C and found that ripening was delayed, as were chlorophyll breakdown and fruit softening. The longer exposure at the highest CO_2 levels resulted in high ethanol and acetaldehyde production rates and resulted in injury, characterized by dark brown discoloration of the epidermis.

There is a significant acceleration of glycolysis and, consequently, an increase in acetaldehyde and ethanol production under anaerobic conditions in most species because mitochondria are less operative (Kennedy *et al.*, 1992). The deficit of high energy metabolites and the change in redox balance in an anaerobic environment requires the plant cell to adapt metabolism in a more or less coordinated way and a shift to energetically inefficient glycolysis may not be the only alternative (Zemlianukhin and Ivanov, 1978). According to these authors running a shortened TCA cycle or the build up of readily mobilizeable substrate, like succinate or γ-aminobutyric acid, are some of the mechanisms that gain more importance under an unbalanced metabolism.

High concentrations of CO_2 in the storage atmosphere have been shown to influence the levels of some intermediates of the TCA cycle by changing the activity of certain enzymes (Kader, 1986). The changes in organic acid concentrations found by several researchers under elevated CO_2 led to the indication that the controlling action of CO_2 was occurring at the TCA cycle (Shipway and Bramlage, 1973). Miller and Hsu (1965) had already indicated that CO_2 concentrations of 15% inhibited the oxidation of several TCA intermediates. Reduced nicotinamide adenine dinucleotide (NADH) and also terminal oxidation inhibition by 15% CO_2 in cauliflower mitochondria were observed by those authors.

Monning (1983) observed that long term exposure of apple tissue to 7.5% CO_2 at 3.5°C resulted in much lower respiratory activity as determined by substrate uptake, and that there was already a noticeable inhibition of metabolism after 3 hours of exposure to 5% CO_2. According to the author, SDH is a very important site for the inhibitory action of CO_2 and, moreover, it appeared that malate dehydrogenase was not affected by CO_2 concentrations.

Although Shipway and Bramlage (1973) verified suppressed oxidation of succinate in apples, their conclusion was that the CO_2 effects could not be solely ascribed to the high sensitivity to CO_2 of the succinoxidase system. The authors considered a more widespread CO_2 effect to be a more likely explanation for reduced mitochondrial activity. The prolonged exposure to CO_2 reduced oxidative phosphorylation and this reduction of the oxidizing capacity was considered to be a consequence of damage to the mitochondria. Further indication of irreversible damage to the mitochondrial structure is derived from an even stronger reduction of oxidative capacity observed after transfer of the fruit to air. The only TCA intermediate whose oxidation was stimulated was malate.

Mitz (1979) suggested that the effects of physiological concentrations of CO_2 at the membrane and molecular levels could collectively be indicative of a sensing and control mechanism. In more extreme concentrations, the CO_2 interactions with proteins imbedded in the lipid bilayer and directly with the membrane lipids may be responsible for changes in the cell environment leading to irreversible damage.

The idea of irreversible damage to the mitochondrial structure caused by elevated CO_2 concentrations was not corroborated by the work of Moriguchi and Romani (1995). These authors reported that isolated mitochondria from avocados subjected to 25 and

75% CO_2 for 43 or 105 hours at 25°C maintained respiratory control (RC) similar to control fruit after transfer to air. In contrast, however, mitochondria isolated from avocados kept under anoxia for the same length of time did not recover RC during the first 4 hours after transfer and there was only little recovery after 6 hours.

Glycolytic enzymes are also affected by elevated CO_2 concentrations in the storage atmosphere (Monning, 1983; Kerbel *et al.*, 1988). Kerbel *et al.* (1988) reasoned that the reduction in O_2 consumption of pears stored in an atmosphere of 10% CO_2 plus air could derive from an inhibitory effect on both PFK and PFP, because PFK is a very important regulatory step in glycolysis. In fact, the authors found that there was an accumulation of fructose-6-phosphate and significantly reduced levels of fructose-1-6-bisphosphate under elevated CO_2, which indicates that this step is affected by elevated CO_2 concentrations.

Hess *et al.* (1993) found that discs of mature avocados subjected to 80% CO_2 plus either 20 or 0.25% O_2 for 2 days at 20°C had reduced activity of PFK, probably mediated, according to the authors, via a drop in cytoplasmic pH produced by dissolved CO_2. Under the same conditions, PFP did not show any activity.

Examining the activity of pyruvate decarboxylase (PDC) and alcohol dehydrogenase (ADH), enzymes that are responsible for the breakdown of pyruvate to ethanol, Chang *et al.* (1983) found that PDC increased significantly along with increasing CO_2 concentrations in sweet potato roots under anaerobic conditions. The authors contended that PDC was at more limiting concentrations than ADH and may, therefore, be regarded as the modulating point of carbon flux from pyruvate towards ethanol.

Ke *et al.* (1995) determined that when O_2 levels were reduced to 0.25% for up to 3 days at 20°C, lactate, acetaldehyde and ethanol concentrations all increased in avocado fruit, which indicates that lactate dehydrogenase (LDH) activity was also stimulated. However, the addition of 80% CO_2 to the low O_2 atmosphere resulted only in the accumulation of acetaldehyde and ethanol, which led the authors to conclude that the high CO_2 atmosphere, by dropping cytoplasmic pH from 6.9 to 6.3, significantly inhibited LDH activity and, therefore, negated the effects of the low O_2 atmosphere.

The observations of Kimmerer and McDonald (1987) that the production rates of acetaldehyde and ethanol are not well correlated derive from the fact that in some species there can be ethanol production, though no detectable acetaldehyde. On the other hand, in some species, for example *Vicia coronata*, there is higher acetaldehyde production compared to ethanol. According to these authors, acetaldehyde is more toxic than ethanol to plant cells, so ADH activity should be more pronounced as a means of detoxification.

Mateos *et al.* (1993) determined increasing concentrations of acetaldehyde and ethanol when storing intact heads or minimally processed lettuce for 10 or 20 days in CO_2 concentrations up to 20% at 2.5°C. The longest exposure of intact lettuce heads to the highest CO_2 concentration resulted in off-flavors detected by informal tasting. Good correlation between off-flavor development and acetaldehyde and ethanol accumulation has been established in several commodities by Ke *et al.* (1991).

After storing strawberries for 9 days at atmospheres of 20 or 50% CO_2 mixed with air, or 0.25% O_2 in 99.5% nitrogen (N_2) at 5°C, Ke *et al.* (1993) concluded that measuring the extractable activity of PDC and ADH did not adequately explain the mode of action of extreme atmospheres on alcoholic fermentation. The authors, based on their own results and published research, suggested three possible mechanisms that could enhance fermentation: enhanced protein synthesis, enzyme activation influenced by a drop in cytoplasmic pH, and higher substrate (pyruvate, acetaldehyde and NADH) availability, favoring the reaction towards ethanol production. Ke *et al.* (1995), working with avocados under very low O_2 and high CO_2 treatments, concluded that increasing concentrations of pyruvate and, subsequently, of acetaldehyde, and the ratio $NADH/NAD^+$ stimulate the ADH reaction, causing ethanol to accumulate.

Yahia *et al.* (1989) found that exposing 'Keitt' mangoes to an atmosphere with 2% O_2 plus 50% CO_2 for a maximum of 5 days at 20°C delayed the respiratory climacteric, but the fruit did not show any external or internal injury. Based on their sensory evaluation, these authors concluded that overall acceptability of CA-treated mangoes was not significantly different from control fruit. These findings are not in accordance with what Lakshminarayana and Subrahmanyam (1970) concluded from their work with 'Alphonso' mangoes stored under CO_2 atmospheres of up to 15% for a maximum of 32 days at 11.1-12.2°C. However, in the experimental set up used by Lakshminarayana and Subrahmanyam (1970), atmospheres initially set at 5, 10 and 15% CO_2 increased to over 20% after a few days. Correspondingly, O_2 levels dropped below 2% and ethanol production rates were at least ten times higher than the rates of control fruit after 21 days under the different CO_2 treatments. Mangoes from the different CO_2 atmospheres were rated unmarketable at the end of the storage periods. However, because monitoring CO_2 concentrations beyond 20% was not possible due to equipment limitations and, moreover, because the O_2 levels were extremely low, it is not possible to regard the original CO_2 settings as atmospheres detrimental for mango storage.

The Effects of CO_2 on Ethylene Biosynthesis

The observation that elevated CO_2 atmospheres drastically reduce ethylene production strongly suggests that the mechanism by which CO_2 delays ripening of fruits stored under CA conditions is mediated via effects on ethylene biosynthesis. With the establishment of the ethylene pathway by Adams and Yang (1979), it has been possible to investigate the effects of different storage conditions on the pathway itself and, furthermore, on the effects on the overall ripening process.

The essentiality of ethylene for fruit ripening has received enormous attention over the years, which led to the determination that a variety of environmental factors influence ethylene production. Among these factors, CO_2 has been identified as an inhibitor of ethylene action. CO_2 has also been reported as having mainly an inhibitory effect on ethylene synthesis of fruits, while in vegetative tissue, generally, there is a promotive effect (Cheverry *et al.*, 1988).

CO_2 has been shown to have different effects on ethylene production that depend on the commodity and factors such as ripeness stage and CO_2 concentration. Aharoni *et al.* (1979) observed an enhancement of ethylene production in tobacco leaf discs exposed to 5 and 10% CO_2. Philosophos-Hadas *et al.* (1986) also observed enhanced ACC oxidase activity *in vivo* in tobacco discs exposed to 10% CO_2. In contrast, however, Philosophos-Hadas *et al.* (1993) concluded that exposure to 10% CO_2 retarded senescence of chervil leaves independently of ethylene action. These authors suggested that the possible mechanism of CO_2 in retarding senescence could derive from its effects on polyamine biosynthesis or metabolism, since the polyamine levels were highest in CO_2

plus norbornadiene (NBD), a potent inhibitor of ethylene action (Sisler and Yang, 1984), in comparison to the control and NBD alone.

Chavez-Franco and Kader (1993) exposed ripe pear discs to CO_2 concentrations from 0.1 to 20% and concluded that any increase in CO_2 concentration suppresses ACC synthase activity, confirming the results of Bufler (1984), who found that CO_2 concentrations over 5% significantly inhibited the enzyme activity from preclimacteric apples. Bufler (1984) also determined that autocatalytic ethylene production was maintained by continued stimulation of ACC synthase activity by ethylene and that the presence of CO_2 inhibited ethylene action, consequently leading to lower stimulation of ACC synthase.

Levin *et al.* (1993) determined that ACC synthase activity was inhibited in 'Jonathan' apples stored for 7 months at 0°C under 10 or 20% CO_2 combined with 3 or 15% O_2. ACC concentrations decreased with increasing CO_2 concentrations. These findings are in accordance with Burg and Burg (1967), who suggested that the accumulation inside the tissues of CO_2 produced by respiratory activity is probably responsible for raising the ethylene threshold level necessary to enable ethylene action.

ACC oxidase, on the other hand, as observed by Chavez-Franco and Kader (1993) in pears, is differentially affected by CO_2. Levels of CO_2 below 5% stimulated the activity of the enzyme, while there was a reduction of activity at 20% CO_2. Levin *et al.* (1993) came to the same conclusions while working with ACC oxidase from 'Jonathan' apples, with the difference that the activity of the enzyme was enhanced by 10% CO_2 but, at 20% CO_2, ACC oxidase activity was inhibited regardless of the O_2 concentration in the storage atmosphere.

Rothan and Nicolas (1994) observed that ethylene production by kiwi pericarp plugs treated with CO_2 concentrations up to 28% decreased with increasing CO_2. However, ACC concentrations remained practically unaffected. These authors also did not find evidence of *de novo* synthesis of ACC oxidase under high CO_2 as had been previously determined by Philosophos-Hadas *et al.* (1986) in tobacco leaf discs, but there seemed to be more of an indication that high CO_2 enhanced the activity of the enzyme *in vivo* when in the presence of saturating levels of ACC.

Poneleit and Dilley (1993) found enhanced activity of ACC oxidase extracted from apples under 5% CO_2 atmosphere. Smith and John (1993) verified the same increasing activity of partially purified ACC oxidase extracted from melons during incubation in CO_2 concentrations up to 20%. In addition, Poneleit and Dilley (1993) also observed that in the presence of 5% CO_2 the activity of ACC oxidase sustained ethylene production for at least 60 minutes. When incubating the enzyme in the presence of ambient CO_2 there was a decrease in the activity over time.

Even though it has been demonstrated by Bufler (1984) that elevated CO_2 concentrations can inhibit ethylene biosynthesis via its effects on ACC synthase, CO_2 is also an essential activator of ACC oxidase (Dong *et al.*, 1992; Fernandez-Maculet *et al.*, 1993; Vioque and Castellano, 1994). Fernandez-Maculet *et al.* (1993) determined that it is CO_2 rather than bicarbonate (HCO_3^-) that is required for ethylene synthesis and that CO_2 exerts its activation on the enzymic reaction by activating the enzyme instead of the substrate. The authors also determined that there is no obligate binding order of CO_2 and ACC to ACC oxidase for the reaction to occur. Fe^{2+} also acts as an activator for ACC oxidase, while O_2 and ascorbate, along with ACC, are substrates for the enzyme (Dong *et al.*, 1992; Moya-Leon and John, 1995).

ACC oxidase has been shown to have CO_2 concentration optima varying with different species and pH. While Dong *et al.* (1992) demonstrated a requirement of 4% CO_2 for maximum *in vivo* activity of the enzyme in apples, Vioque and Castellano (1994) found that optimal ACC oxidase activity from pears was at a CO_2 concentration of 14%. Mizutani *et al.* (1995) found that the optimum pH for ACC oxidase from apple is 7.4 at ambient CO_2 and, like Dong *et al.* (1992), found that maximal enzyme activity is attained at 4% CO_2. However, they found that maximal ACC oxidase activity at 20% CO_2 occurred at pH 6.7. Poneleit and Dilley (1993) indicated a pH optimum of 6.8 for apple ACC oxidase with and without supplemental CO_2.

Controlled Atmosphere and Aroma Volatiles of Mangoes

In addition to the effects on ethylene biosynthesis, extreme CO_2 concentrations also have pronounced effects on aroma volatiles. There has been extensive research in evaluating reduced volatile production under long term CA storage of temperate fruit species. Streif and Bangerth (1988) showed that in apples, the higher the CO_2 concentrations and the lower the O_2 concentrations in the CA atmospheres, the more suppressed the production of aroma volatiles. Ultimately, low aroma negatively influences the flavor (Streif, 1978; Williams and Langron, 1983).

Storage time is also a factor that influences volatiles. Willaert *et al.* (1983) demonstrated that in apples there is a significant decrease in the total amount of released volatiles following long term CA of more than 6 months compared to short term CA of about 3 months. Moreover, the authors determined that apples from the shorter storage period reached the volatile production peak faster than the apples from long term CA.

The same strategy used to extend storage life of deciduous fruit crops, *i.e.*, harvest at preclimacteric stages to limit ripening during CA and in that way prolong storage, has been employed for extending mango storage life. Ripe mangoes deteriorate much faster than mangoes harvested at the mature green stage (Thomas and Joshi, 1988), but unquestionably have better flavor characteristics than the latter ones.

Flavor characteristics of mangoes are an important aspect for consumer acceptance (Engel and Tressl, 1983). Despite high popularity of mangoes, at least in tropical areas, there has been limited research to characterize and quantify aroma components (MacLeod and Snyder, 1985). Mango aroma profiles vary in the intensity and in the quantity of individual compounds (Ackerman and Torline, 1984; Koulibaly *et al.*, 1992) and each recognized group of cultivars has characteristically different profiles (MacLeod and Pieris, 1984). There is not much agreement on which group of compounds is the most important amongst the volatiles, but it is generally recognized that concentrations may vary from about 60 µg/kg to 230 mg/kg and that the volatiles mainly derive from lipid metabolism (Koulibaly *et al.*, 1992).

Quality of mangoes upon retrieval from different storage treatments has been mainly designated using descriptive terms or by using numeric scores based on hedonic scales (Lakshminarayana, 1980). There have been no systematic determinations of the most important aroma components under different postharvest conditions. A series of publications have examined a diverse number of mango cultivars from different growing areas and quantitative and qualitative differences have become clear. MacLeod and

Snyder (1985) examined two Florida cultivars, ‘Keitt’ and ‘Tommy Atkins’, and compared their profiles to Indian type cultivars and concluded that it is possible to establish five different compounds as the most important for flavor, which emphasizes the importance of the genetic material in influencing aroma.

Engel and Tressl (1983) characterized 114 compounds in the ‘Alphonso’ and ‘Baladi’ cultivars, of which 85 were identified for the first time in mangoes. These two cultivars have distinct sensory properties described by the authors as a green, fresh and fruity note for ‘Alphonso’ and a mild, caramel-like and reminiscent of melon note for ‘Baladi’.

Bartley and Schwede (1987) analyzed the aroma profile of ripe ‘Bowen’ mango and determined that only two components, from a total of 40, were responsible for 72% of the total volatile organic material. The two major compounds, ethyl butanoate and terpinolene, together are not paralleled in another cultivar. Comparing the profiles of overripe fruit to the profiles of fresh ripe fruit, the authors verified a significant decrease of ethyl butanoate, which is supposed to be the major contributor to the unique flavor of fresh from this particular cultivar.

MacLeod and Snyder (1985) studied volatiles of ‘Tommy Atkins’ and ‘Keitt’ mangoes and concluded that the profiles for both cultivars are very similar. Both produced similar amounts of volatiles, about 72 μg/g for ‘Tommy Atkins’ and 54 μg/g for ‘Keitt’, and had car-3-ene as the most abundant compound. When examining the changes of the aroma compounds of ‘Keitt’ mangoes as ripening progresses, the authors found that there was little change in the total concentration of volatiles, but there were some progressive changes, a decrease in sesquiterpene hydrocarbons, especially βcaryophyllene, compared to an increase in esters. A striking component of the overripe fruit was the occurrence of significantly high amounts of alcohols, principally ethanol at about 47.5% w/w of the total volatiles. With the increasing amounts of alcohols, other important compounds decreased drastically, with the car-3-ene concentration dropping more than 50% and limonene having even greater reduction in concentration. Storage of slices from tree ripe 'Keitt' mangoes in polyethylene film for 13 days at 10°C did not prevent the loss of flavor compounds. As a matter of fact, the stored slices were similar to the overripe mangoes, yet alcohols were present in even greater concentration. Ethanol represented about 90% w/w of the total volatiles.

CHAPTER 3

MANGO TOLERANCE TO REDUCED OXYGEN PLUS ELEVATED CARBON DIOXIDE LEVELS

Introduction

Mangoes are increasing in popularity among consumers in more developed countries. There have been reports showing yearly increases of about 10% in import volumes to the United States during part of the last two decades (Haines, 1991). These increasing amounts of fruit have shifted the transport means from air cargo to marine shipments. Marine transport is less expensive and also better accommodates the higher volumes of fruit (Medlicott *et al.*, 1990).

The disadvantage of marine shipments is the time that a vessel takes to reach consumer markets (Noomhorn and Tiasuwan, 1995). It might take from 2 to 3 weeks from the Caribbean, or Mexico or South America to arrive in Europe or the U.S. This amount of time takes its toll on fruit quality. Although the mangoes arrive in marketable condition there is still room for quality improvements. One of the alternatives could be harvest at riper stages combined with shipment under CA.

Also, the hot water treatment currently used for disinfestation from the Caribbean fruit fly (*Anastrepha suspensa*) can result in quality losses (Jacobi and Wong, 1991; Mitcham and McDonald, 1992). Alternatively, there have been attempts to use either elevated CO_2 or reduced O_2 as quarantine treatments. Yahia *et al.* (1989) reported that a five day exposure of 'Keitt' mangoes to 50% CO_2 at 20°C did not cause injuries. With the present experiments, in contrast to short term CO_2 exposure, the objective was to examine the possibility of longer term CA storage of mangoes under high CO_2 concentrations.

Material and Methods

Two experiments evaluating extreme CO_2 atmospheres were conducted during the summer of 1993. Freshly harvested mature green 'Tommy Atkins' mangoes from the Homestead, Florida area and 'Kent' mangoes from the Pine Island/Fort Myers, Florida area were transported by car to the Horticultural Sciences Department facilities in Gainesville. Harvest was based on ground color and fruit size and shape. Individual fruit were placed in 1.75 l glass jars on the day of harvest and held in a flow-through system with different combinations of O_2 and CO_2. The gases for the mixtures were delivered by cylinders of compressed CO_2, O_2 and nitrogen (N_2). The following treatments were applied to the mangoes: 5%, 25%, 50% and 70% CO_2 combined with either 3% or 20.8% (air level) O_2 for 14 days for 'Tommy Atkins' and for 21 days for 'Kent' mangoes. Control fruit were held in air in the flow through system. Storage temperature was 12°C.

A third experiment was conducted in the summer of 1994 with mature green 'Tommy Atkins' mangoes imported from Mexico by Brooks Tropicals in Homestead, Florida and transported by refrigerated truck to Gainesville. These mangoes were handled under normal commercial conditions, treated with 46°C water for 60 minutes for insect disinfestation, and coated with a commercial formulation of carnauba wax prior to shipment to Florida by refrigerated truck. These mangoes were stored at 12°C in atmospheres of 15%, 25%, 35% and 45% CO_2 combined with 5% O_2 for 21 days. In this experiment, O_2 levels were obtained by mixing air delivered by an air compressor with CO_2 and N_2. The control treatment was storage in air in the same system.

During the storage period, 0.5-ml headspace samples were retrieved from each of the four replicates/treatment and injected into gas chromatographs (GC) to analyze for ethanol and ethylene production. For the determination of respiration rates (CO_2 production) the gas inlet and outlet of each replicate were sealed for 2 hours. One 0.5-ml headspace sample was retrieved immediately after sealing the jar and a second sample was taken after 2 hours and respiration rates calculated as described in Talasila *et al.* (1992). After the storage period, the mangoes were transferred to 20°C for 5 more days. During the 5 days of ripening at 20°C, the jars containing the mangoes were sealed for 1 hour and 0.5-ml headspace samples were retrieved from each replicate and analyzed for ethanol, ethylene and CO_2 by GC.

Ethanol was determined on a Hewlett-Packard GC (Avondale, PA), model 5890, equipped with a 152x3.12 mm 80/120 mesh Carbopack B (5% Carbowax 20M) column at 140°C. The flame ionization detector (FID) and injector were set at 200°C. Ethylene was determined on a photoionization GC, Photovac 10A10 (Thornhill, Ontario, Canada), equipped with a 76x3.18 mm 60/80 activated alumina column. There is no temperature control on the Photovac 10A10 and column temperature rises as a consequence of heat from the UV source of the detector. The injector is at room temperature. CO_2 levels were determined on a Fisher Gas Partitioner (Pittsburgh, PA), model 1200, equipped with a 196.6x3.18 mm 80/100 mesh Porapak Q column at 60°C. The thermal conductivity detector and injector temperatures were set at 90°C.

Epidermal ground color was measured using a portable Minolta chromameter (Ramsey, NJ), model CR200, calibrated with a white standard plate, CRA43. Two measurements from each fruit were taken at random on the distal end. Hue angles and chroma were calculated from the 'a' and 'b' values according to McGuire (1992).

Fruit firmness determinations were performed only on the mangoes of the last experiment of this series of experiments using a Cornell firmness device (Hamson, 1952) as modified by Gull (1987). Two measurements per fruit were taken on opposing equatorial sides, without removing the epidermis. A 1 kg mass was applied and deformation in mm was recorded after 5 seconds.

The experiments were conducted in a completely randomized design. Statistical analysis of the data was done by using SAS for PC (SAS Institute Inc., Cary, NC).

Results and Discussion

Mangoes stored under high CO_2 atmospheres had higher ethanol production rates compared to control fruit during the same storage period. After storage for 21 days, 'Kent' mangoes in 50% or 70% CO_2 plus either 3% or air levels of O_2 did not differ in ethanol production but did differ significantly from control fruit (Figure 3.1A). 'Tommy Atkins' mangoes stored for 14 days showed the same trend in terms of ethanol production (Figure 3.2), though the absolute values of ethanol were not as high. Ethanol production by the mangoes stored in 5 and 25% CO_2 was significantly lower than the production rates in the higher CO_2 atmospheres in these experiments (Figures 3.1B, 3.2).

The same trend was observed with the mangoes during CA storage in the third experiment of this series. Fruit in the maximum CO_2 treatment, 45% CO_2 for 21 days, had significantly higher ethanol production rates (Figure 3.3). Mangoes exposed to 35% CO_2, however, had ethanol levels only slightly different from the control fruit and fruit exposed to 15 or 25% CO_2.

Lakshminarayana and Subrahmanyam (1970) reported similar increases in ethanol production in 'Alphonso' mangoes at 11.1-12.2°C after 21 days in elevated CO_2 atmospheres in comparison to control fruit. Likewise, O'Hare and Prasad (1993) also observed an increase in ethanol in 'Kensington' mangoes along with CO_2 injury in CO_2 concentrations over 10% for a maximum of 5 weeks at 7°C. They concluded that those fruit were easily identifiable because of off-odors and skin discoloration. In the present experiments, darkened skin areas were only visible in CO_2 treatments of 45% and higher.

Ke *et al.* (1990) also found that pears exposed to 50 or 80% CO_2 for 10 days at 0, 5 or 10°C had significantly higher ethanol production rates compared to the air control or 20% CO_2. These authors also observed that 80% CO_2 differed from the 50% CO_2 treatment and that ethanol production rates remained higher after transfer to air at 20°C following prolonged exposure to CO_2. This increase in ethanol production was also observed in this study with 'Kent' and 'Tommy Atkins' mangoes after transfer to air at 20°C (Figures 3.1 and 3.2). Ethanol production by control fruit also increased during ripening at 20°C, though the levels remained much lower than that of fruits from the CA treatments.

There was a dramatic decrease in ethanol production after transfer to air from both the higher CO_2 concentrations of the last experiment in this series (Figure 3.3), but the mangoes from the 45% CO_2 treatment still had significantly higher ethanol production than the mangoes from the other treatments at the end of the 5-day ripening period.

Ethanol production rates of control fruit and those from the 15 and 25% CO_2 treatments remained at the same levels as during storage.

A decrease in ethanol production 7 days after transfer to air was also observed by Li and Kader (1989) in strawberries stored for 7 days at 2°C in combinations of 0, 1 or 2% O_2 and 10, 15, or 25% CO_2. Even though ethanol production increased in control strawberries in air, the levels remained significantly lower than those of the combinations of 0.5% O_2 plus 20% CO_2 and 1% O_2 plus 15% CO_2.

'Kent' and 'Tommy Atkins' mangoes did not show significant differences in ethanol production between the two O_2 levels used (Figures 3.1 and 3.2), except for the 3% O_2 plus 25% CO_2 treatment of 'Tommy Atkins' mangoes, where ethanol production reached levels similar to the 50 and 70% CO_2 atmospheres. Contrasting 3% with air levels of O_2 indicated that the O_2 concentration did not significantly influence ethanol production (Figures 3.1, 3.2, and 3.3). This could be an indication that extreme CO_2 concentrations overshadow the effects of low O_2 in mangoes, considering that it has already been indicated in other species, for example avocados (Kanellis *et al.*, 1989b) and pears (Ke *et al.*, 1994), that O_2 levels below 5% induce ADH isozymes, thus enhancing ethanol production.

Respiration rates of 'Kent' mangoes in the 50 and 70% CO_2 treatments were significantly higher during CA storage compared to control fruit (Figure 3.4A) while the mangoes from the 5 and 25% CO_2 treatments did not differ from control fruit (Figure 3.4B). After transfer to air at 20°C, respiration rates of control fruit and fruit stored in 5 or 25% CO_2 increased about twofold while the respiration rates of 50 or 70% CO_2-exposed fruit dropped significantly below control levels, which could indicate irreparable damage to the tissues. ‘Tommy Atkins’ mangoes stored at 50 and 70% CO_2 for 14 days also showed a drop in CO_2 production after transfer to air at 20°C for 5 days (Figure 3.5). The lower CO_2 atmospheres of 5 and 25% CO_2 showed the increase and subsequent drop in CO_2 production typical of non-damaged tissues after transfer to air at 20°C. There were no differences in CO_2 production during CA storage that could be related to O_2 concentrations.

After transferring to air at 20°C, ‘Tommy Atkins’ mangoes stored for 21 days in CO_2 concentrations up to 45% initially showed no significant differences in respiration rates (Figure 3.6). There was, however, a difference in the subsequent patterns of CO_2 production between 15% CO_2 and 45% CO_2, which probably is evidence of some damage to the respiratory system by the 45% CO_2 treatment. All the other treatments seemed to have regained respiratory control after 5 days in air since their patterns of CO_2 production were similar to control fruit.

The high respiration rates and high ethanol production of mangoes stored at CO_2 levels above 25% clearly indicate intensive anaerobic metabolism, which also was perceived by off flavors during informal taste tests. The mechanism by which high CO_2 concentrations resulted in anaerobiosis probably involves inhibition of enzymes of the TCA cycle, especially succinate dehydrogenase (Monning, 1983; Kubo *et al*., 1990). Moreover, as indicated by Ke *et al.* (1995), when electron transport and oxidative phosphorylation are inhibited, the only source of ATP for tissue survival is through substrate phosphorylation in the glycolytic pathway, which depends on the availability of reducing power (NAD^+).

The continuous increase in ethanol and decrease in CO_2 production of CA mangoes from the 50 and 70% CO_2 atmospheres after transfer to air may indicate irreversible damage to the mitochondrial structure and its inability to recover respiratory control after transfer to air following prolonged exposure to extreme CO_2 atmospheres. Even though Moriguchi and Romani (1995) found that avocado mitochondria were able to regain respiratory control after short term exposure to 75% CO_2, the prolonged storage of mangoes in 50% and 70% CO_2 might have had the same effect as short term exposure to anoxia on the mitochondria from avocados, which did not completely recover respiratory activity after transfer to air.

Mangoes exposed to 25% CO_2 for up to 2 weeks at 12°C recovered respiratory activity similar to air control and 5% CO_2 fruit after transfer to air at 20°C, which might indicate that the electron transport system in fruit from these treatments was not completely shut down during CA storage and also was not severely damaged by the treatment. Ke *et al.* (1995) have observed that the electron transport system in avocados in 80% CO_2 plus air levels of O_2 for 3 days at 20°C was only partially inhibited compared to the same level of CO_2 plus 0.25% O_2. In the treatment with 80% CO_2 plus 0.25% O_2, the $NADH/NAD^+$ ratio was significantly higher than the ratio in fruit from 80% CO_2 plus air levels of O_2, which was similar to the ratio of control fruit. Decreased availability of oxidized reducing potential is one of the mechanisms by which ethanol production is stimulated (Ke *et al.*, 1991; 1995).

Ethylene production rates of control mangoes of both cultivars were significantly higher than the production rates of mangoes held in 50 or 70% CO_2, both during storage (data not shown) and after transfer to air (Figures 3.7 and 3.8). Although the 50 and 70%

CO_2 atmospheres shifted the metabolism towards anaerobiosis, causing damage to the mangoes as indicated by high ethanol production, ethylene production did not increase as would be expected under a stressful condition. This could indicate that high CO_2 concentrations may have inhibited ethylene synthesis as previously shown for other fruits (Cheverry *et al.*, 1988; Kerbel *et al.*, 1988; Rothan and Nicolas, 1994). The significantly lower ethylene production of 'Kent' and 'Tommy Atkins' mangoes in 50 and 70% CO_2 may be ascribed, according to Rothan and Nicolas (1994), to the effects of CO_2 on ACC oxidase. However, Dong *et al.* (1992) have shown that CO_2 is an essential activator of ACC oxidase and therefore leads to increased ethylene production *in vitro*. Mathooko *et al.* (1995), on the other hand, have shown that a 60% CO_2 plus 20% O_2 treatment for 24 hours stimulated ethylene production along with enhanced O_2 uptake in cucumbers. During the exposure time to CO_2, the authors determined significant increases in the activities of ACC oxidase and ACC synthase as well, but 12 hours after transfer to air, enzyme activity and ethylene production dropped to control levels. Despite these observations, it is possible that the concentrations of 50% CO_2 and higher for 2 or 3 weeks of storage for mangoes were above stimulatory levels and caused permanent damage to tissues and to the ethylene biosynthetic pathway, since ethylene production remained low after transfer to air.

The O_2 levels used in these experiments seemed to have little effect on the ethylene synthesis in either mango cultivar. Although O_2 is required for the oxidation of ACC to ethylene (Yang, 1985), 3% O_2 could be higher than the apparent K*m* for ACC oxidase in mango, using as an analogy the K*m* of 0.4% O_2 determined by Kuai and Dilley (1992) in apples. It seems that the high CO_2 concentrations were primarily responsible for inhibition of ethylene production in our experiments. The mangoes from both cultivars in the 5 and 25% CO_2 atmospheres had ethylene production patterns during CA storage that were similar to control fruits, though the rates of production were significantly lower (data not shown). After transfer to air, ethylene production of 'Kent' mangoes from the 3% O_2 or air plus 5 or 25% CO_2 treatments had significantly higher ethylene production than control fruit (Figure 3.7). These higher ethylene production rates could indicate a stress response even though the respiration rates (Figure 3.4) did not indicate so. Ethylene production of these treatments in 'Tommy Atkins' mangoes (Figure 3.8), on the other hand, did not differ from control fruit.

'Tommy Atkins' mangoes stored in atmospheres with up to 45% CO_2 for 3 weeks in the third experiment resumed ethylene production after transfer to air at 20°C (Figure 3.9). The recovery of ethylene production in this experiment, despite the physical injury discussed below, may indicate that not all metabolism is impaired under CO_2 concentrations between 25 and 45% CO_2. Moreover, it might also indicate that irreparable damage occurs if mangoes are stored for more than 2 weeks in an atmosphere with more than 50% CO_2. However, ethylene production was generally lower in the later experiment with 'Tommy Atkins' compared to the earlier experiment. This difference may have been due to inhibition of ethylene production following the hot water treatment used for insect quarantine in the Mexican mangoes used for the later experiment.

At the beginning of storage, the mangoes were predominantly green, as indicated by high hue angles. During the storage period, all CO_2 treatments significantly delayed decreases in hue angles (yellowing) compared to control fruit (Figure 3.10), which is probably a consequence of reduced chlorophyll breakdown influenced by CO_2 as reported by Ke *et al.* (1990) in pears after short term storage in 20, 50 or 80% CO_2. Through 21 days of storage, all the CO_2-treated mangoes had similar hue angle values, close to 100°, which denotes an essentially green ground color. During the 5 days after transfer to air at 20°C, however, the 25% CO_2-treated fruit showed a very intense color change so that the hue angles were not statistically different from control fruit, while the 45% CO_2-stored fruit still had hue angles over 90°, indicating that these mangoes underwent minimal chlorophyll degradation, which could be an indication of CO_2 damage. The 15 and 35% CO_2-treated mangoes, although with hue angles below 90°, did not differ significantly from the 45% CO_2 treatment. However, it is possible that there are different mechanisms leading to the similar hue angles in the fruit from the 15 and 35% CO_2 treatments. Under the more extreme atmospheres, 35 and 45% CO_2, the damage or stress caused by CO_2 to either enzymes or membranes may have affected chlorophyll degradation. These treatments increased ethylene production compared to the control after transfer to air at 20°C (Figure 3.9), which may be an indication of a stress response or injury to the tissues resulting in an inability to develop normal coloration. The 25% CO_2 treatment had ethylene production rates similar to the higher CO_2 atmospheres, which could have enhanced chlorophyll degradation during the ripening period at 20°C, leading to hue angles similar to control fruit but without the detrimental effects inflicted by the higher CO_2 atmospheres. On the other hand, the 15% CO_2 treatment had an irregular pattern of ethylene production (Figure 3.9) and, therefore, lagged behind in chlorophyll breakdown.

Similar to the patterns of changes in hue angles, the chroma values also changed little during CA storage (Figure 3.11). Control fruit had significantly higher chroma values compared to CO_2-stored fruit. After transfer to air at 20°C, chroma values remained the lowest in the 45% CO_2 treatment, which was not significantly different from the 15 and 35% CO_2 treatments, while mangoes from the 25% CO_2 treatment did not differ from control fruit after 5 days in air. The fruit from the 45% CO_2 treatment had an abnormal, dull, grayish-green appearance, probably resulting from the conversion of chlorophylls to pheophytins. In thermally processed green vegetables the chlorophylls are converted to pheophytins by the loss of the Mg^{2+} ions changing the color from bright green to olive-green (Pither, 1993; LaBorde and von Elbe, 1994). Steele and Johnson (1995) determined the same changes in color in processed kiwifruit. The authors suggested that the drop in pH was responsible for the degradation of chlorophylls to pheophytins, which confer a more brown color to the processed product.

Chroma values and hue angle determinations could be somewhat deceptive because the mango pigments with significant changes during ripening, chlorophylls, carotenoids and anthocyanins (Laksminarayana, 1980; Medlicott *et al.* 1986), may have been affected differently by the CO_2 treatments. Examining 'a' and 'b' values (Table 3.1) shows that there is no considerable difference in their patterns from those seen with either hue or chroma. The 'a' and 'b' values in the 35 and 45% CO_2 atmospheres were significantly lower compared to the other treatments 5 days after transfer to air. This could indicate, to some extent, less chlorophyll breakdown, but could also reflect the occurrence of browning rather than maintenance of ground color close to the values at harvest. The same misleading results have been observed in strawberries by Nunes *et al.* (1995), where seemingly contradictory lower hue and 'a' values did not indicate brighter red color but rather a less attractive reddish brown color of the berries.

Table 3.1: 'a' and 'b' values of 'Tommy Atkins' mangoes at transfer from storage during 21 days in air, 15, 25, 35 and 45% CO_2 at 12°C and after 5 days in air at 20°C.

	'a' value		'b' value	
	At transfer	+5 days in air	At transfer	+5 days in air
Air	6.38	14.76	43.79	44.13
15% CO_2	- 5.48	2.02	32.59	39.05
25% CO_2	- 6.38	9.18	33.77	42.90
35% CO_2	- 8.45	2.28	29.86	39.19
45% CO_2	- 4.22	- 1.68	32.77	36.92
LSD < .05	5.70		4.60	

Finally, the intense change in the 'a' value of the 25% CO_2 treatment after transfer to air is noteworthy. The massive change in 'a' value in the 25% CO_2 treatment may give more support to the idea that enhanced chlorophyll degradation stimulated by increased ethylene production occurred, mainly during the first days after transfer to air, which was not the case in the 15% CO_2 treatment. This is in agreement with the conclusions of Pesis *et al.* (1994), who considered that exposure of 'Golden Delicious' apples to 95% CO_2 for up to 48 hours had a stimulating effect on ethylene production and, therefore, enhanced yellowing of the apples during the following 2 weeks in air at 20°C.

Epidermal color differences as a consequence of prolonged storage of mangoes under elevated CO_2 atmospheres have previously been observed by O'Hare and Prasad (1993), MacLauchlan and Barker (1994) and Noomhorn and Tiasuwan (1995). Although the CO_2 concentrations used in those experiments were lower, a maximum of 10% CO_2, all the authors determined that the color development had been retarded by CO_2 treatments during storage. Short term exposure to 50% CO_2 for 24 hours at 20°C has also been reported by Yahia *et al.* (1989) to delay ripening and color changes in 'Keitt' mangoes.

Mango flesh softening as measured by mm of deformation did not seem to be very substantially delayed during long term CO_2 treatments (Figure 3.12). All the treatments were significantly softer after 5 days in air compared to the time of transfer with control fruit showing the largest increase in deformation. Fruit from the 15 and 45% CO_2 treatments were less firm than control fruit at the time of transfer, but after 5 days in air at 20°C the 25, 35 and 45% CO_2 treatments were firmer than the control, while the 15% CO_2 treatment was not significantly different from the control. The positive effect of the CO_2 treatments in delaying softening during ripening at 20°C could derive from reduced polygalacturonase (PG) activity, as has been observed by Pesis and Marinanski (1993) after pretreating tomatoes with 70% CO_2 for 24 hours. Yahia *et al.* (1989) also reported that a 50% CO_2 plus 2% O_2 treatment for 84 hours at 20°C resulted in much firmer mango fruit 12 days after transfer to air compared to the air control.

Even though there was a tendency to more resistance to deformation with increasing CO_2 concentrations, the retention of firmness in the 35 and 45% CO_2 treatments probably was a result of damage to the tissues and truncated ripening. On the other hand, in the 15% CO_2 treatment deformation was comparable to the control fruit, and, when considered with the lower color development (Figures 3.10 and 3.11), resulted in a greener yet softer mango, which is not in keeping with consumer preferences (Lakshminarayana, 1980). On the other hand, fruit from the 25% CO_2 treatment retained firmness equal to the 35 and 45% CO_2 treatments while developing more normal color like that of the control fruit.

Conclusions

The storage of mangoes in CO_2 atmospheres above 25% resulted in increased respiration rates, increased ethanol levels and reduced ethylene production rates. Reduction of O_2 from air levels to 3% in the presence of elevated CO_2 had little additional effect on ethanol and ethylene production. Levels of CO_2 of 35% and higher caused abnormal color development. Mango resistance to deformation during ripening in air at 20°C was enhanced by elevated CO_2 atmospheres.

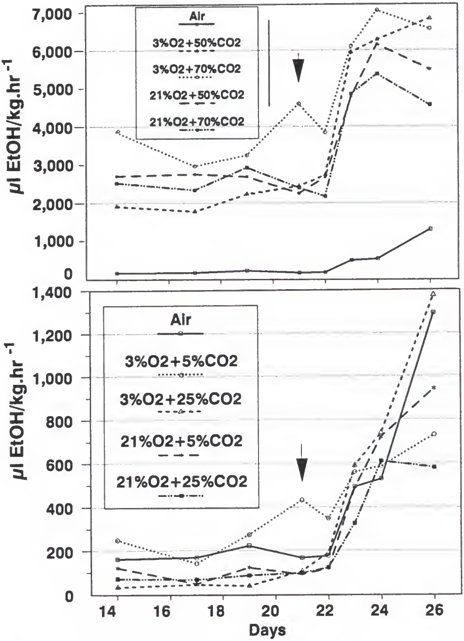

Figure 3.1: Ethanol production by 'Kent' mangoes stored for 21 days in CA or air at 12°C plus 5 days in air at 20°C. Arrow indicates transfer to air. (Vertical bar = LSD p<.05).

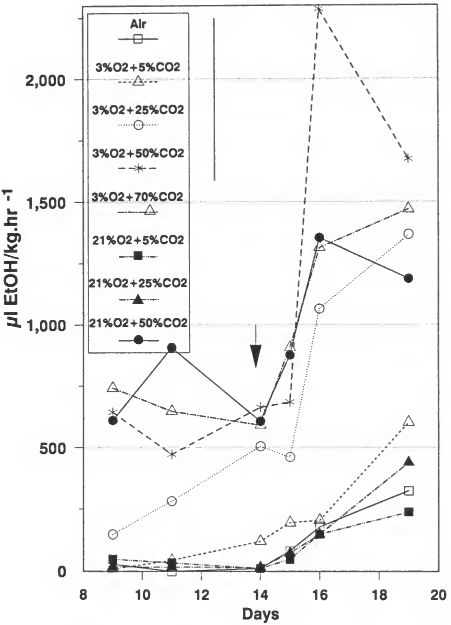

Figure 3.2: Ethanol production by 'Tommy Atkins' mangoes stored for 14 days in CA or air at 12°C plus 5 days in air at 20°C. Arrow indicates transfer to air. (Vertical bar = LSD p<.05).

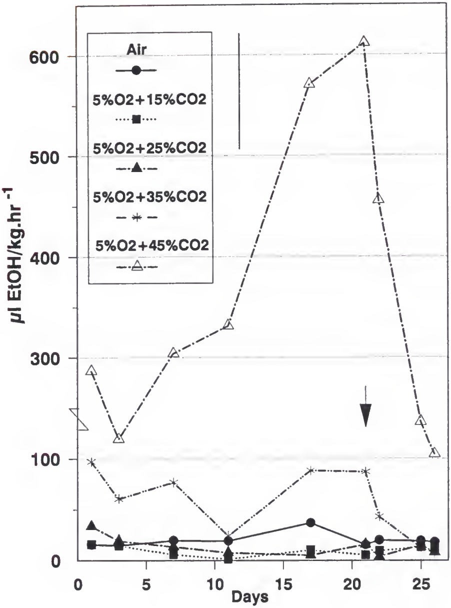

Figure 3.3: Ethanol production by 'Tommy Atkins' mangoes stored for 21 days in CA or air at 12°C plus 5 days in air at 20°C. Arrow indicates transfer to air. (Vertical bar = LSD p<.05).

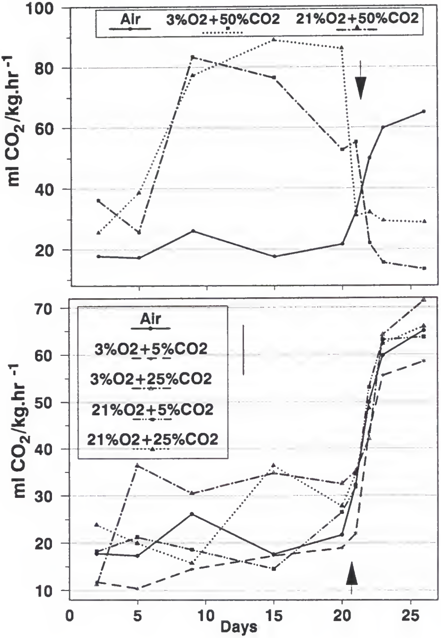

Figure 3.4: Respiration rates of 'Kent' mangoes stored for 21 days in CA or air at 12°C plus 5 days in air at 20°C. Arrow indicates transfer to air. (Vertical bar = LSD p<.05).

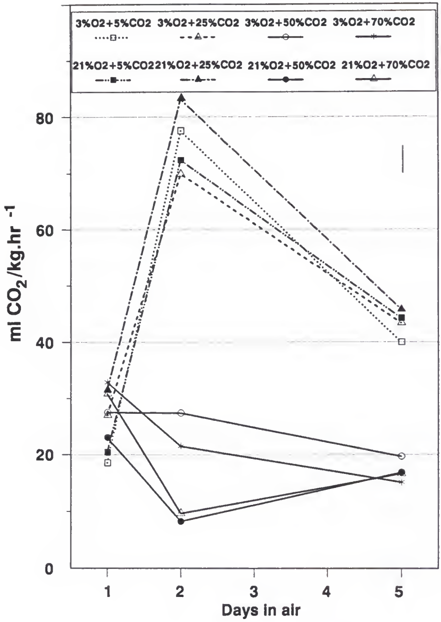

Figure 3.5: Respiration rates of 'Tommy Atkins' mangoes stored in air at 20°C for 5 days following storage in CA or air for 14 days at 12°C. (Vertical bar = LSD p<.05).

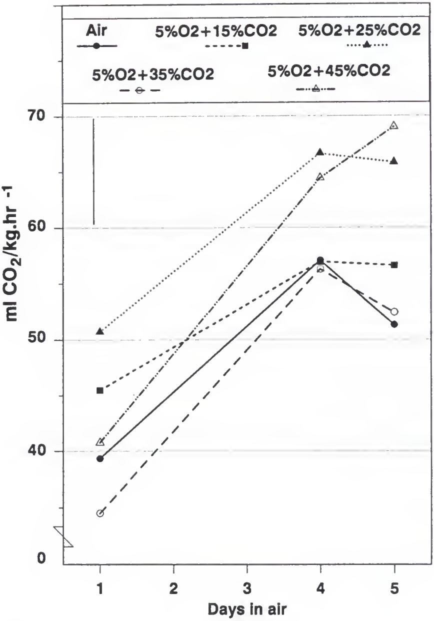

Figure 3.6: Respiration rates of 'Tommy Atkins' mangoes stored in air at 20°C for 5 days following storage in CA or air for 21 days at 12°C. (Vertical bar = LSD p<.05).

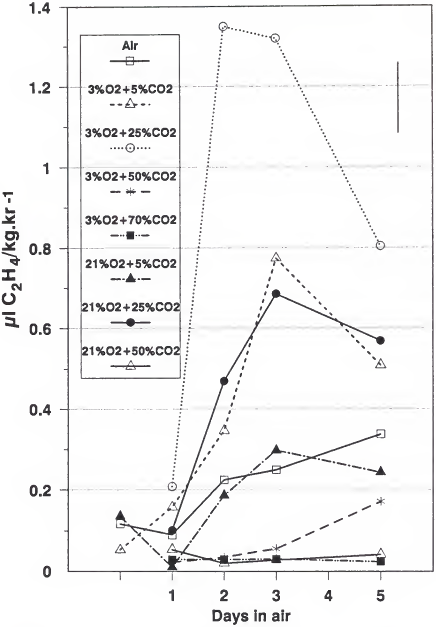

Figure 3.7: Ethylene production by 'Kent' mangoes stored in air at 20°C for 5 days following storage in CA or air for 21 days at 12°C. (Vertical bar = LSD p<.05).

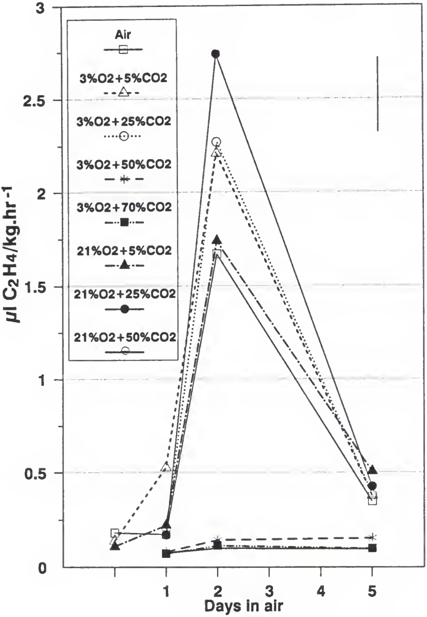

Figure 3.8: Ethylene production by 'Tommy Atkins' mangoes stored in air at 20°C for 5 days following storage in CA or air for 14 days at 12°C. (Vertical bar = LSD p<.05).

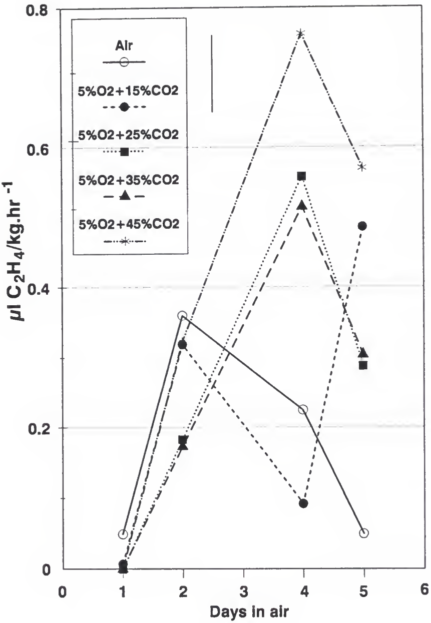

Figure 3.9: Ethylene production by 'Tommy Atkins' mangoes stored in air at 20°C for 5 days following storage in CA or air for 21 days at 12°C. (Vertical bar = LSD p<.05).

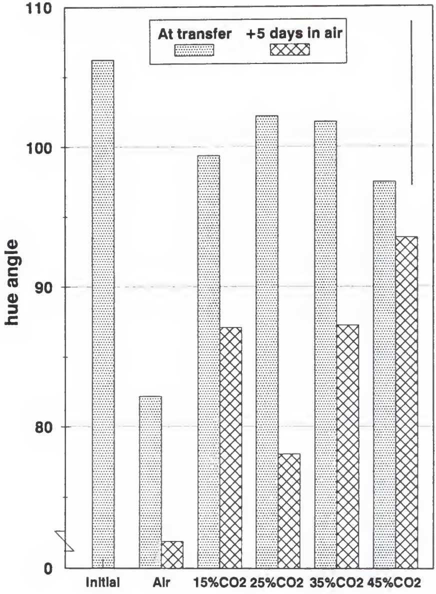

Figure 3.10: Hue angles of 'Tommy Atkins' mangoes stored for 21 days in CA or air at 12°C plus 5 days in air at 20°C. (Vertical bar = LSD p<.05).

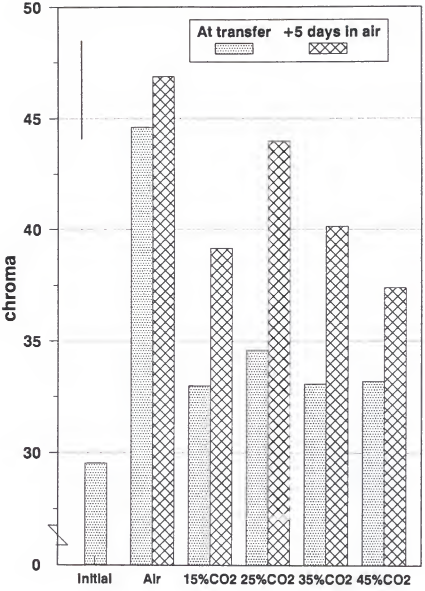

Figure 3.11: Chroma values of 'Tommy Atkins' mangoes stored for 21 days in CA or air at 12°C plus 5 days in air at 20°C. (Vertical bar = LSD p<.05).

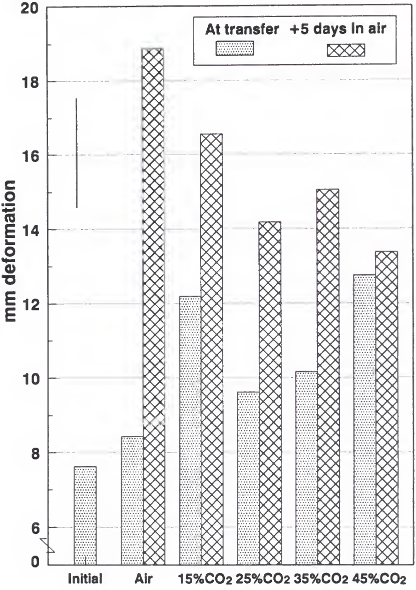

Figure 3.12: Firmness of 'Tommy Atkins' mangoes measured as mm deformation after 21 days in CA or air storage at 12°C plus 5 days in air at 20°C. (Vertical bar = LSD p<.05).

CHAPTER 4
LOW OXYGEN ATMOSPHERES FOR MANGO STORAGE

Introduction

Many beneficial effects of CA storage are attributed to the reduction of O_2 concentrations. Reduced respiratory activity in bananas (Kanellis *et al.*, 1989a) and apples (Gran and Beaudry, 1993), slower softening rates in apples (Nichols and Patterson, 1987), papaya (Yahia *et al.*, 1992), pears (Ke *et al.*, 1990) and strawberries (Li and Kader, 1989), slower ground color breakdown in bananas (Wills *et al.*, 1982) and in 'Granny Smith' apples (Little *et al.*, 1982), maintenance of higher organic acid levels in pears (Yoshida *et al.*, 1986) and in apples (Little and Peggie, 1987) are some of the positive results observed over the course of the years deriving from low O_2 atmospheres.

In contrast to these positive results, undesirable responses have been observed when examining the applicability of more extreme O_2 and/or CO_2 atmospheres to extend storage life, for the alleviation of physiological disorders, and for insect disinfestation or postharvest pathogen control (Ke *et al.*, 1995). These undesirable responses consist mainly of development of off-flavors as the tissues shift to glycolytic reduction of pyruvate to ethanol (Leblova, 1978; Kader, 1986; Gran and Beaudry, 1993). It is well established that under hypoxia, meaning limited O_2 availability, the activities of PDC and ADH increase (Kimmerer, 1987; Nanos *et al.*, 1992), leading to the accumulation of acetaldehyde and ethanol (Kennedy *et al.*, 1992).

The O_2 concentration at which the transition from aerobic to anaerobic respiration occurs has been termed by Boersig *et al.* (1988) as the anaerobic compensation point (ACP). Further studies to determine the lowest O_2 concentration at which anaerobic respiration would still be absent or avoided has indicated that the ACP, at least in some species, is strongly influenced by physiological age of the commodity (Boersig *et al.*, 1988) and by the storage temperature (Beaudry *et al.*, 1992). Moreover, tolerance to low O_2 environments is species dependent (Kennedy *et al.*, 1992) and the tolerance to various exposure times is characteristic for each commodity (Ke *et al.*, 1993).

In mangoes, only recently has tolerance to hypoxia started to be evaluated in the search for insecticidal atmospheres for fruit fly disinfestation. Yahia *et al.* (1989) reported that a 3-day, 2% O_2 treatment at 20°C did not cause injury to 'Keitt' mangoes. No noticeable off-flavors were detected, but lower acceptability ratings were given by sensory panelists to the low O_2-treated fruit. Yahia and Hernandez (1993) concluded that mangoes from the same cultivar tolerated a 0.5% O_2 treatment at 20°C for 5 days. Mangoes from this treatment did not produce detectable off-flavors and besides, were firmer and greener than control fruit kept in air at the same temperature.

In the present experiments, as opposed to short term, insecticidal low O_2 treatments, the objective was to determine the response of mangoes to the reduction of O_2 concentrations in the storage atmosphere for longer term CA. More specifically, it was intended to determine the O_2 concentration at which anaerobic respiration is enhanced and to evaluate fruit ripening during and after transfer from CA.

Material and Methods

Mature green 'Haden' mangoes from Peru and 'Tommy Atkins' mangoes from Brazil used in these experiments were imported by Brooks Tropicals in Homestead, Florida, and transported by refrigerated truck to Gainesville. These mangoes were handled, at the origin, under normal commercial conditions, treated with 46°C water for 60 minutes for insect disinfestation, and coated with a commercial formulation of carnauba wax prior to refrigerated marine shipment to Florida. Upon arrival, the mangoes were selected for freedom from defects and immediately placed in glass jars and transferred to the designated storage room.

Individual 'Haden' fruit were placed in 1.75-l glass jars, six replicates per treatment, and held in a flow-through system under the different concentrations of O_2 at 15°C for 14 days. Air, delivered by a compressor, was mixed with N_2 from a cylinder to produce the following treatments: 2, 3, 4, and 5% O_2. One control treatment was air in the flow-through system and a high CO_2 control was obtained by combining 25% CO_2 with air. The same treatments were applied to 'Tommy Atkins' mangoes held in 10.05-l glass jars for 21 days at 12°C. In this experiment, four fruit per replicate and four replicates per treatment were used.

During the storage period, 0.5-ml headspace samples were retrieved from each replicate and injected into GC to analyze for ethanol and ethylene production and respiration rates (CO_2 production), as described in Chapter 3. After the storage period, the mangoes from each experiment were transferred to air at 20°C for 5 days. During the 5 days of ripening, the fruit were sealed in the jars and, after 1 hour, 0.5-ml headspace samples were retrieved for ethanol and ethylene production and respiration rate determinations.

Epidermal color determinations were made at transfer from CA and after 5 days in air using a portable Minolta chromameter, model CR200, calibrated to a white standard plate, CRA43. Two random measurements were taken at the distal end and one measurement on the shaded side at the equator of each fruit.

Flesh color was only determined with 'Tommy Atkins' mangoes. Two measurements were taken on opposing equatorial sides of each fruit after removing the epidermis. Hue angles and chroma were calculated from the 'a' and 'b' values according to McGuire (1992).

Fruit firmness determinations of 'Haden' mangoes were performed using a Cornell firmness device (Hamson, 1952) as modified by Gull (1987). Two measurements per fruit were taken on opposing equatorial sides, without removing the epidermis. A 1 kg mass was applied and deformation in mm was recorded after 5 seconds.

Flesh firmness of 'Tommy Atkins' mangoes was determined using an Instron Universal Testing Machine (Canton, MA), model 1132, equipped with a 50-kg load cell and an 11-mm diameter convex Magness-Taylor test probe. Two measurements were taken on opposing equatorial sides of each fruit after removal of the epidermis. After the firmness measurements, the fruit were peeled and mesocarp tissue homogenized for the determinations of total sugars, titratable acidity, pH and starch contents.

Titratable acidity was determined by titration of 6.00 g mango juice diluted in 50 ml deionized water with 0.1N NaOH to a pH endpoint of 8.2 using an automatic titrimeter, Fisher Scientific (Pittsburgh, PA), model 381. The pH of the juice was determined with a pH meter. Total sugars were determined colorimetrically via the phenol-sulfuric method as described in Dubois *et al.* (1956). Starch content was determined from the alcohol insoluble material by overnight enzymic digestion at 37°C with α-amylase and amyloglucosidase, followed by measurement of the resulting soluble sugars by the phenol-sulfuric method as described in Bergsma and Brecht (1992).

The experiments were conducted in a completely randomized design. Statistical analysis of the data was done using SAS for PC (SAS Institute Inc., Cary, NC).

Results and Discussion

Respiration rates in both cultivars were lower in the low O_2 treatments compared to control fruit (Figures 4.1 and 4.2). After transfer to air at 20°C, CO_2 production in all the treatments showed a similar sharp increase in respiratory activity. The pattern of ethylene production in both cultivars was similar to the pattern of CO_2 production, with control fruit producing more ethylene than fruit from the CA treatments (Figures 4.3 and 4.4). After transfer to air, ethylene production increased significantly, but, again, there were no differences in the pattern between treatments.

Reduced respiratory activity and ethylene production under low O_2 atmospheres have been observed in commodities such as strawberries (Li and Kader, 1989), bananas (Kanellis *et al.*, 1989a), pears (Nanos *et al.*, 1992) and bell peppers (Rahman *et al.*, 1993). In all these species, respiratory activity remained lower after transfer to air. This residual effect of low O_2 on the respiratory activity was considered by Kanellis *et al.* (1989b) as deriving, primarily, from the inhibition of ethylene action by low O_2 and not from restriction of the activity of oxidases. Rahman *et al.* (1993), on the other hand, working with bell peppers that were transferred to air after a 24-hour storage under 1.5% O_2 at 20°C, suggested that the residual response could be derived from the suppression of extramitochondrial oxidases rather than from the suppression of the mitochondrial, high-affinity for O_2, cytochrome oxidase.

In mangoes, only fruit from the 'Haden' cultivar showed a residual effect of the reduced O_2 atmosphere. Ethylene production and respiration rates of the two lowest O_2 treatments remained lower than the rates of control fruit after transfer to air at 20°C, but, after 5 days in air, the differences were minimal. In 'Tommy Atkins', no residual effect of the low O_2 atmosphere was determined, which could be attributed to cultivar differences in response to low O_2 concentrations. Differences in ripeness stage between the cultivars could also account for the differences in behavior. The 'Tommy Atkins' mangoes were riper than the 'Haden' mangoes when entering CA storage, as indicated by the epidermal hue angles: 103.4 ± 2.8 for 'Tommy Atkins' compared to 119 ± 1.8 for 'Haden'.

Despite being initially greener than the 'Tommy Atkins' fruit, 'Haden' control fruit underwent a significant color change during the 2 weeks of storage at 15°C (Figures 4.5 and 4.6). Mangoes from the O_2 treatments during the CA period showed a concentration response for both hue and chroma. Hue and chroma of the 25% CO_2 treatment remained similar to the values of the lowest O_2 treatment. This could be an indication that inhibition of ethylene action is a possible mechanism involved in the delay of epidermal color development in CA-stored mangoes based on observations by Burg and Burg (1962), who determined that color development in 'Kent' and 'Haden' mangoes was synchronous with increasing ethylene production during the climacteric peak. The same concurrence has been observed by Krishnamurthy and Subrahmanyam (1970) working with 'Pairi' mangoes and by Cua and Lizada (1990) working with 'Carabao' mangoes.

Further indication that the inhibition of ethylene action by low O_2 atmospheres is, at least partially, accountable for color development can be derived from the hue angles of 'Tommy Atkins' mangoes (Figure 4.7). 'Tommy Atkins' mangoes were at the onset of the climacteric (Figure 4.2) at the beginning of storage and continued to produce ethylene (Figure 4.4) at levels slightly higher than those of 'Haden' mangoes (Figure 4.3) even though stored at a lower temperature. These initial ethylene levels might not have been enough to trigger color development. The subsequent prevention of ethylene action by low O_2 atmospheres might have been able to delay pigment changes in the epidermis during the 21 day CA period of 'Tommy Atkins' mangoes. Tian *et al.* (1994) determined that broccoli florets changed hue angles in an ethylene dose-dependent decline. The authors determined that an ethylene concentration as low as 0.01 ppm for 36 hours at 20°C was enough to induce a 20% decrease in hue angle of the florets. Mangoes, in contrast to broccoli, have low levels of ethylene production (Burdon *et al.*, 1996) and, while the fruit of the low O_2 treatments in the present experiments were exposed to ethylene concentrations of about 0.01 $\mu l\ C_2H_4/kg.hr^{-1}$ for the duration of storage at 12°C, *i.e.*, 14 and 21 days, changes in hue angles during this period were less than 10% of the initial value, which could indicate that either ethylene action was suppressed or that mangoes have an higher ethylene threshold level for color development.

After transfer from low O_2 storage to air at 20°C, there was an intense color change in both cultivars. However, in almost all the treatments, the color of the low O_2 fruit did not reach the values of the control fruit. The most evident differences were observed in the fruit stored at 2% O_2 and at 25% CO_2, where hue angles in both cultivars (Figure 4.5 and 4.7) remained significantly higher than the values of control fruit. As for chroma values, the most striking suppression was observed in the 25% CO_2 treatment. These differences in the response of color development to low O_2 and high CO_2 point to the various pigments involved in mango epidermal color and their specific responses to different storage conditions, as already indicated by Lakshminarayana (1980) and Medlicott *et al.* (1986).

Flesh color of 'Tommy Atkins' mangoes (data not shown) was not affected by the CA treatments. There was also no effect of storage time on the flesh color. At transfer from CA to air, the averages for all the treatments plus control fruit were 57.03 ± 1.65 for chroma values and 90.37 ± 0.68 for hue angles. After 5 days in air, the averages were 56.93 ± 1.84 and 90.28 ± 1.02 for chroma and hue, respectively.

Ethanol production rates in both 'Haden' (Figure 4.9) and 'Tommy Atkins' (Figure 4.10) increased with decreasing O_2 concentrations in the storage atmosphere. The lowest O_2 treatment produced the highest levels of ethanol. The 5% O_2 and 25% CO_2 treatments had ethanol production rates similar to control fruit. The 3% O_2 treatment also showed enhanced ethanol production in both cultivars. Even though there was slightly enhanced ethanol production in the 4% O_2 treatment, it did not differ significantly from control fruit during CA storage. Ethanol production increased significantly after transfer to air at 20°C in all the treatments, but remained the highest in the 2% O_2 treatment.

The elevated ethanol production in 'Haden' and 'Tommy Atkins' mangoes in the low O_2 treatments may be attributed to the upregulation of ADH isozymes as a response to low O_2 as has been observed by Ke *et al.* (1994) in pears and by Ke *et al.* (1995) in avocados. Ethanol production during CA storage of 'Tommy Atkins' mangoes was more than twofold the production of 'Haden' mangoes. In a preliminary experiment, 'Haden' mangoes that were at the onset of the climacteric at the beginning of storage (Figure 4.11A) had much higher ethanol production rates during CA than fruit of the same cultivar that entered CA storage at a less ripe stage (Figure 4.1), remaining preclimacteric during the 2-week storage period, while the fruit in Figure 4.11 went through the climacteric rise in respiration and ripened during storage. Thus, besides cultivar differences, ripeness stage also seems to positively influence ethanol production.

Ethanol has been determined to be a component of mango flavor and has been detected in many cultivars (Ackerman and Torline, 1984). An increase in ethanol production by control fruit during ripening was detected in all the different cultivars and in all the experiments of the present work. This increase has also been observed by MacLeod and Snyder (1985) when examining aroma profiles of 'Keitt' mangoes. The authors determined that the relative abundance of ethanol in the total volatile spectrum increased dramatically from green (0.3%) to overripe fruit (47.5%) kept at room temperature, even though they did not consider ethanol as a genuine aroma component of fresh mangoes since it was detected only in stored or overripe fruit.

Quality parameters of 'Haden' (Table 4.1) and 'Tommy Atkins' (Table 4.2) mangoes had intensive changes during ripening for 5 days at 20°C. There were significant changes in all the variables analyzed over the course of the 5-day ripening period.

Mangoes of both cultivars softened extensively during storage at 12°C and after transfer to air. Excessive fruit softening is a very important aspect of postharvest quality maintenance of mangoes and might significantly reduce the storage potential if the fruit are

Table 4.1: Quality parameters of 'Haden' mangoes stored for 14 days in air or CA at 15°C plus 5 days in air at 20°C.

	Starch (% F. W.)		Titratable acidity (% citric acid)		Total sugars (mg/g F. W.)		Firmness (mm deformation)	
	At transfer	+5 days/air	At transfer	+5 days/air	At transfer	+5 days/air	At transfer	+5 days/air
Air	0.062	0.042	0.83	0.61	47.76	31.45	11.5	14.0
2% O_2	0.066	0.045	2.84	2.03	55.74	49.54	11.1	12.9
3% O_2	n.d./[1]	0.044	n.d.	1.68	n.d.	36.72	9.1	12.0
4% O_2	n.d.	0.044	n.d.	1.28	n.d.	46.55	10.3	11.3
5% O_2	0.048	0.052	1.93	0.86	41.18	68.16	9.5	13.6
25% CO_2	0.048	0.039	1.80	1.38	55.25	45.46	11.2	12.8
LSD p<.05	NS/[2]		0.31		NS		2.1	

/[1] n.d. = not determined.

/[2] NS = only a significant day effect.

Table 4.2: Quality parameters of 'Tommy Atkins' mangoes stored for 21 days in air or CA at 12°C plus 5 days in air at 20°C.

	Starch (% F. W.)		Titratable acidity (% citric acid)		Total sugars (mg/g F. W.)		Firmness (Newton)	
	At transfer	+5 days/air	At transfer	+5 days/air	At transfer	+5 days/air	At transfer	+5 days/air
Air	0.056	0.056	0.54	0.36	188.08	93.78	5.15	4.90
2% O_2	0.055	0.039	1.18	1.14	187.68	76.40	10.02	4.82
3% O_2	n.d./[1]	n.d.	1.48	1.02	182.34	93.04	8.15	7.89
4% O_2	n.d.	n.d.	0.95	0.81	n.d.	78.72	8.40	5.51
5% O_2	0.058	0.058	1.09	0.60	118.30	87.51	8.17	11.53
25% CO_2	0.048	0.046	1.12	0.75	198.26	106.21	12.26	10.17
LSD p<.05	NS/[2]		0.32		37.70		NS	

/[1] n.d. = not determined.

/[2] NS = only a significant day effect.

harvested at a riper stage (Medlicott *et al.*, 1990). Even though both 'Haden' and 'Tommy Atkins' entered CA storage at a mature green ripeness stage, as indicated by ethylene production (Figures 4.1 and 4.2) and ground color (Figures 4.5 and 4.7), the degree of softening, determined either as deformation or mesocarp tissue rupture force, was extensive, with control fruit showing the lowest resistance to deformation compared to the high CO_2 and low O_2 treatments. Ledger (1991) determined that 'Kensington' mangoes are at eating ripeness when flesh firmness values reach 4 Newton (N). Seymour *et al.* (1990) determined that 'Kent' mangoes harvested at the mature green stage had firmness values below 4 N after 14 days at 12°C. Tucker and Seymour (1991) related differences in the degree of mango softening among different cultivars at different ripeness stages to differences in cell wall structure and to the variability in cell wall degrading enzymes and their response to ethylene. Mitcham and McDonald (1992) concluded that in addition to polygalacturonase activity, other enzymatic and non-enzymatic processes are responsible for the extensive softening that occurs during mango ripening, which leads to the conclusion that the ethylene threshold to activate the onset of softening must be very low or there are other mechanisms involved in firmness loss in mangoes.

Although starch breakdown was extensive, it was not completed during the ripening period in both 'Haden' and 'Tommy Atkins' mangoes as reported by Lakshminarayana (1980), Medlicott *et al.* (1986) and Lizada (1994) for fruit that had attained eating quality. The starch levels found in the present experiment, however, are only a fraction of the 11% of starch for mature green freshly harvested 'Julie' mangoes reported by Sankat *et al.* (1994). Selvaraj *et al.* (1989) determined that starch content varied from 0.9 to 4.2% in 7 Indian type mango cultivars. In these fruit the starch content ranged from 0.38 to 0.67% after 6 days of ripening at 25°C.

There was a significant decrease in total sugars during the 5 days of ripening at 20°C in 'Tommy Atkins' fruit, but no significant changes in sugar levels in 'Haden' fruit (Tables 4.1 and 4.2). Medlicott and Thompson (1985) showed that 'Keitt' mangoes had two- to threefold greater total sugars after ripening for 7 days at room temperature. This seems to indicate that conversion of starch to sugars was complete by the end of the storage period in the experiments reported here.

Titratable acidity of 'Haden' and 'Tommy Atkins' mangoes was significantly influenced by the CA treatments and acidity levels significantly decreased during the ripening period at 20°C. Krishnamurthy and Subrahmanyam (1970), Medlicott and Thompson (1985), and Vazquez-Salinas and Lakshminarayana (1985) all reported more intensive decreases in acid levels during mango ripening than observed here. After 5 days in air at 20°C, titratable acidity was significantly higher in the 25% CO_2 and all the O_2 treatments except for the 5% O_2 atmosphere compared to control fruit. A decrease in acids, accompanied by little change in total sugars may indicate that the acids are the main respiratory substrate in the postharvest life of mangoes. However, lower total sugar contents after 5 days in air at 20°C, as determined with 'Tommy Atkins' fruit, could indicate that glycolysis was running unhindered, causing an accumulation of pyruvate. Pyruvate accumulation could derive from reduced oxidative phosphorylation because of less energy demand, which finally limits the carbon flux through the TCA cycle. Increased amounts of pyruvate may then stimulate its own reduction to ethanol as indicated by Ke *et al.* (1993; 1995). This could explain the accumulation of ethanol observed in these experiments without corresponding elevated CO_2 production that would be expected if anaerobiosis was induced. Lower sugar contents could also result from slow replenishing of hexoses via partially hindered starch hydrolysis. Even though amylases are present during mango ripening (Fuchs *et al.*, 1989), there might be signaling molecules downstream responsible for feedback regulation of amylases or hexokinases, in turn controlling hexose availability to enter the glycolytic pathway as has been observed by Sheen (1990) using isolated maize mesophyll protoplasts.

Conclusions

The 2% O_2 treatment caused a residual effect in ethylene production and respiratory activity in 'Haden' mangoes but with only a slight positive effect on quality parameters. There was a significant decrease in titratable acidity after transfer to air in all treatments. However, starch levels were low at transfer to air at 20°C and did not change thereafter, while sugar levels either did not change in 'Haden' or declined in 'Tommy Atkins'. Fruit from the 25% CO_2 treatment behaved similarly to the control treatment except that epidermal color development was significantly reduced by the 25% CO_2 treatment during storage and after transfer to air at 20°C. The 2% O_2 treatment also inhibited epidermal color development, especially hue angles. The effects of 25% CO_2 and 2% O_2 on epidermal color were likely due to inhibition of ethylene action by low O_2 and high CO_2. Hue and chroma values of mesocarp tissue of 'Tommy Atkins' mangoes were not influenced by any of the treatments. Reducing the O_2 concentrations in the storage atmosphere did not induce higher CO_2 production typical of anaerobic metabolism. Storage for 2 weeks at 15 or 12°C in O_2 concentrations of 2 and 3% led to enhanced ethanol production in 'Haden' and 'Tommy Atkins' mangoes.

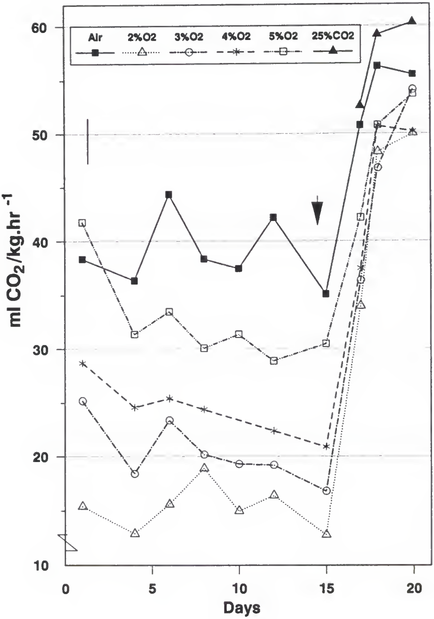

Figure 4.1: Respiration rates of 'Haden' mangoes stored in CA or air for 14 days at 15°C plus 5 days in air at 20°C. Arrow indicates transfer to air. (Vertical bar = LSD p<.05).

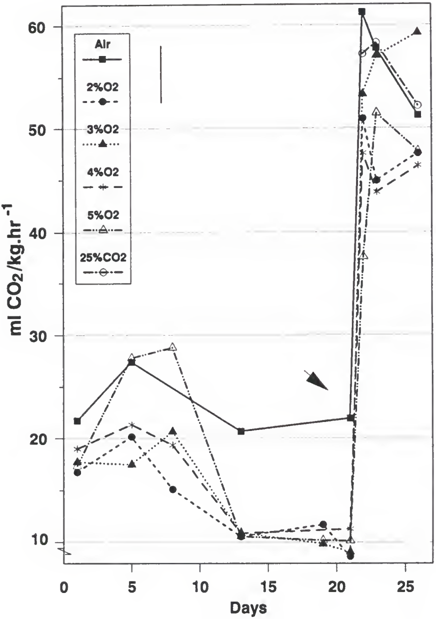

Figure 4.2: Respiration rates of 'Tommy Atkins' mangoes stored in CA or air for 21 days at 12°C plus 5 days in air at 20°C. Arrow indicates transfer to air. (Vertical bar = LSD p<.05).

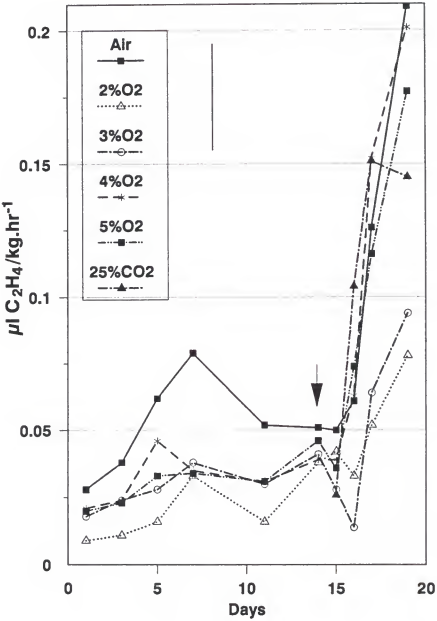

Figure 4.3: Ethylene production of 'Haden' mangoes stored in CA or air for 14 days at 15°C plus 5 days in air at 20°C. Arrow indicates transfer to air. (Vertical bar = LSD p<.05).

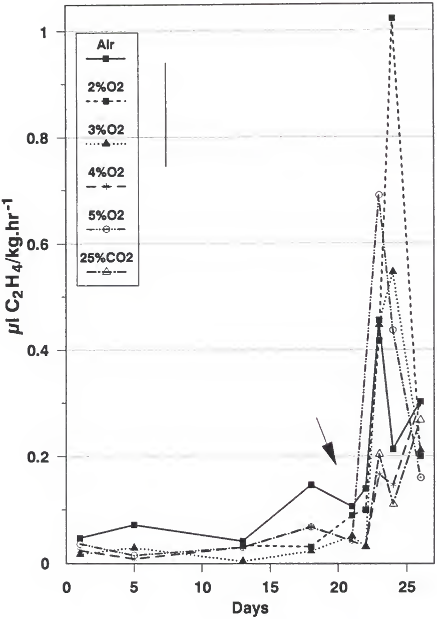

Figure 4.4: Ethylene production of 'Tommy Atkins' mangoes stored in CA or air for 21 days at 12°C plus 5 days in air at 20°C. Arrow indicates transfer to air. (Vertical bar = LSD p<.05).

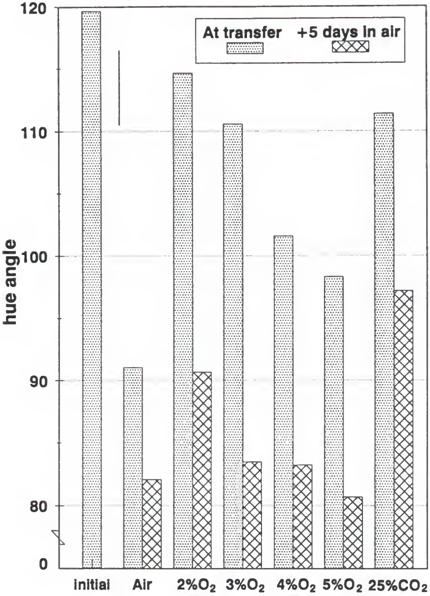

Figure 4.5: Hue angles of 'Haden' mangoes stored in CA or air for 14 days at 15°C plus 5 days in air at 20°C. (Vertical bar = LSD p<.05).

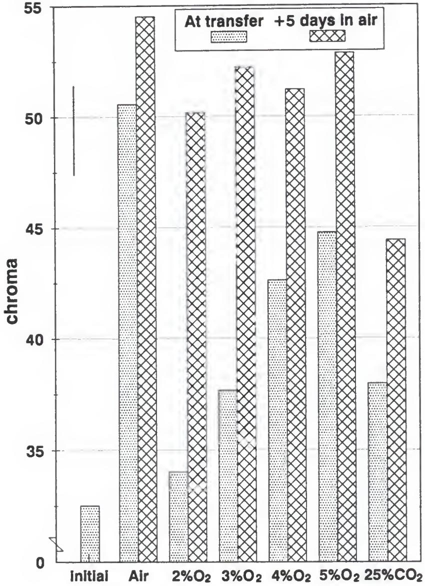

Figure 4.6: Chroma values of 'Haden' mangoes stored in CA or air for 14 days at 15°C plus 5 days in air at 20°C. (Vertical bar = LSD $p<.05$).

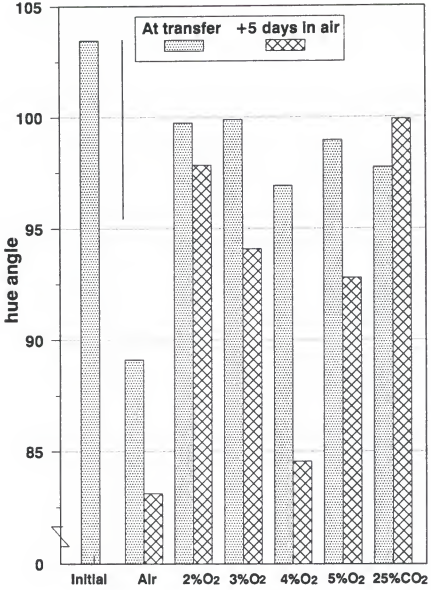

Figure 4.7: Hue angles of 'Tommy Atkins' mangoes stored in CA or air for 21 days at 12°C plus 5 days in air at 20°C. (Vertical bar = LSD p<.05).

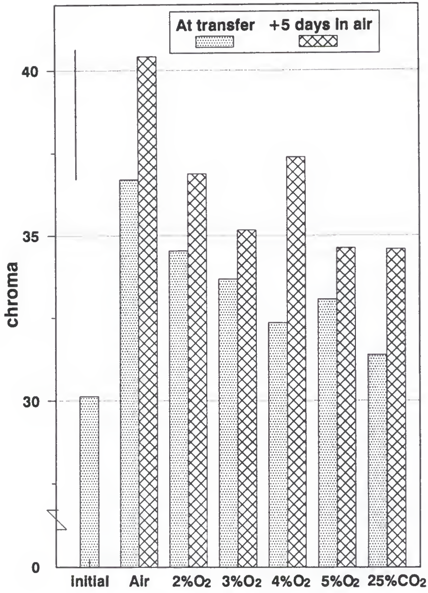

Figure 4.8: Chroma values of 'Tommy Atkins' mangoes stored in CA or air for 21 days at 12°C plus 5 days in air at 20°C. (Vertical bar = LSD p<.05).

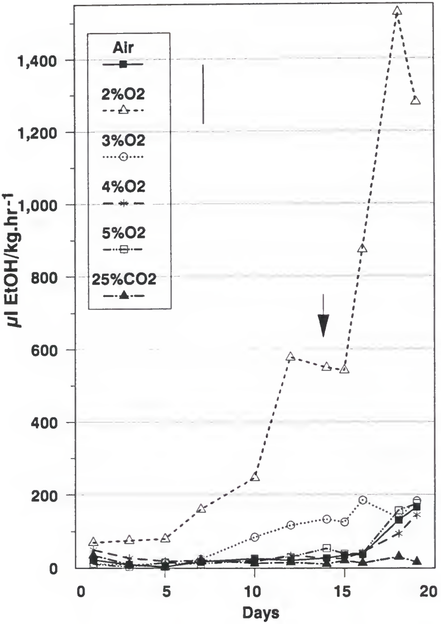

Figure 4.9: Ethanol production of 'Haden' mangoes stored in CA or air for 14 days at 15°C plus 5 days in air at 20°C. Arrow indicates transfer to air. (Vertical bar = LSD p<.05).

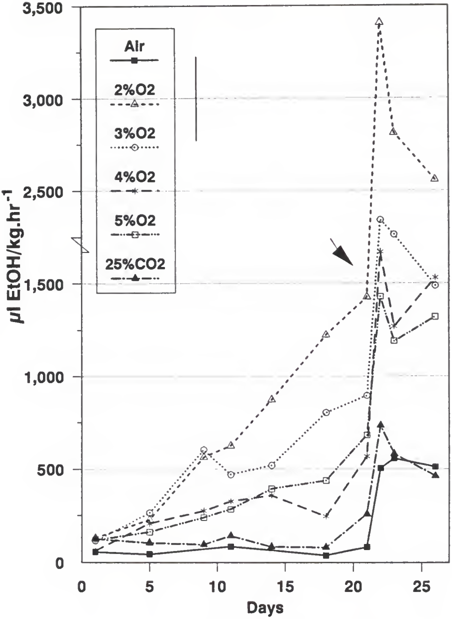

Figure 4.10: Ethanol production of 'Tommy Atkins' mangoes stored in CA or air for 21 days at 12°C plus 5 days in air at 20°C. Arrow indicates transfer to air. (Vertical bar = LSD p<.05).

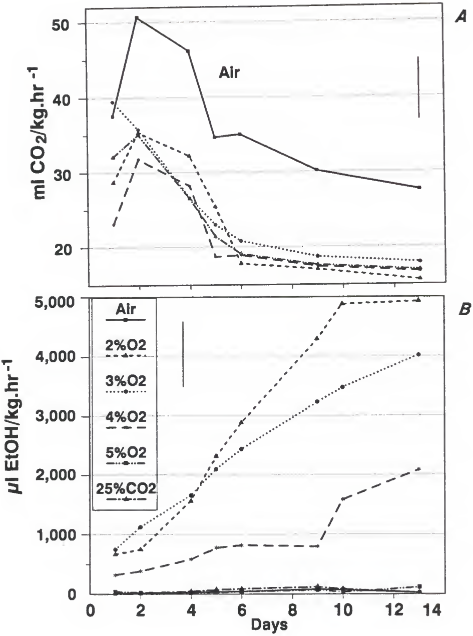

Figure 4.11: Respiration rates (*A*) and ethanol production (*B*) of 'Haden' mangoes stored in CA or air for 13 days at 15°C. (Vertical bar = LSD p<.05).

CHAPTER 5
REDUCING TEMPERATURE FOR CA STORAGE OF TREE RIPE MANGOES

Introduction

The occurrence of chilling injury (CI) in mango storage has always been of great concern as a limitation on the use of temperature management to maintain quality and extend storage life. A wide range of temperatures and factors, such as exposure time, ripeness stage and cultivars, have been reported to significantly influence the manifestation of CI symptoms (Lyons, 1973; Veloz *et al.*, 1977; Chaplin *et al.*, 1986; Thomas and Joshi, 1988; Medlicott *et al.*, 1990; O'Hare and Prasad, 1993). The recommended temperature for mature green mango storage is 12-13°C (Medlicott *et al.*, 1987; Kader, 1993), but even at this temperature Veloz *et al.* (1977) observed occurrence of CI.

Many different symptoms have been described as deriving from storage at chilling temperatures. Among those, uneven ripening, sunken lesions and browning of the epidermis, pale yellow flesh color, and lower resistance to fungal diseases are the most common (Thomas and Oke, 1983; Farooqi *et al.*, 1985; Chaplin *et al.*, 1991; O'Hare and Prasad, 1993).

Alleviation of CI in mangoes has been accomplished by atmosphere modification (Veloz *et al.*, 1977; O'Hare and Prasad, 1993). Chaplin *et al.* (1986) significantly reduced the severity of skin browning in some mango cultivars by storage in low-density polyethylene bags at 1°C. Under these conditions, the authors determined that O_2 levels were below 10% and CO_2 above 5% after 2 days in storage. O'Hare and Prasad (1993) determined that 5 or 10% CO_2 atmospheres alleviated CI symptoms in 'Kensington' mangoes stored for 21 days at 5°C. In addition, Chaplin *et al.* (1991) concluded that 'Kensington' mangoes, in which ripening processes had already started, were substantially less sensitive to chilling temperatures than mangoes harvested at the mature green ripeness stage.

Based on these conclusions and also on some of the observations by Medlicott *et al.* (1989), Seymour *et al.* (1990) and Ledger (1991), amongst others, that mangoes harvested at the ripe stage did not have as long a storage life in cold storage as mangoes harvested at the mature green stage, the first objective of the present work was to determine whether harvesting mangoes at an advanced ripeness stage and storing them in CA at temperatures below the recommended level could enhance storage life and maintain better quality. Another objective was to examine the viability for CA storage with elevated CO_2 concentrations to enhance the storage capability of mangoes at temperatures below the threshold for CI.

Material and Methods

The mature green and tree ripe 'Tommy Atkins' mangoes were harvested at a grove of Brooks Tropicals in Homestead, Florida, while the tree ripe 'Keitt' mangoes were harvested at Gulf Island Grove from Pine Island/Fort Myers, Florida. Selection for harvest was based on ground color and fruit shape, *i.e.*, the outgrowing of the shoulders as described in Medlicott *et al.* (1989). Initial values for hue and chroma are presented in the tables in the discussion. The mangoes were transported by car to Gainesville the day after harvest and, upon arrival, the fruit were classified for ripeness stage, randomly assigned to treatments, and placed in the cold rooms.

Two experiments were conducted with 'Tommy Atkins' mangoes stored for 21 days. In the first experiment, mature green or tree ripe fruit were placed in 10.05-l glass jars and the following treatments, three replicates per treatment, applied in the flow through system: 10, 25 or 45% CO_2 combined with 5% O_2. The control treatment was air, also in the flow through system. Storage temperature for this experiment was 12°C. The second experiment was also conducted with three replicates in the flow through system with the following treatments: 10 or 25% CO_2 combined with 5% O_2. The control treatment was air in the flow through system. Besides eliminating the 45% CO_2 treatment in this experiment, tree ripe fruit were also stored at 8°C under the same atmospheres.

The third experiment was conducted with 'Keitt' mangoes stored for 21 days. In this experiment, only tree ripe mangoes were used. Upon arrival in Gainesville, the mangoes were immersed for 3 minutes in 53°C water containing 1000 ppm tiabendazole. The fruit were separated into two sets: one stored at 5°C and the other at 8°C. Treatments, with four replicates per treatment, were the same as in the second experiment.

A fourth experiment was conducted with 'Tommy Atkins' mangoes, imported from Mexico by Brooks Tropicals in Homestead and transported by car to Gainesville. These mangoes, harvested at the mature green ripeness stage, had been handled under normal commercial conditions, treated wit 46°C water for 60 minutes for insect disinfestation, and coated with a commercial formulation of carnauba wax prior to shipment to Florida by refrigerated truck. This experiment repeated the same settings as the third experiment, but the storage time was only 14 days, with no transfer to air at 20°C.

A 0.5-ml headspace sample was retrieved from each replicate every 3 or 4 days and injected into a gas chromatograph (GC) for ethanol and ethylene determination as described in Chapter 3. After 21 days, the mangoes were transferred to air at 20°C and ethylene and ethanol together with respiration rates (CO_2 production) were determined.

At transfer from CA and after 5 days in air for 'Tommy Atkins', and after 3 days in air for 'Keitt' mangoes, epidermal color determinations were made using a portable Minolta Chromameter (Ramsey, NJ), model CR200, calibrated to a white standard plate, CRA43. Mesocarp tissue color measurements were also taken from 'Keitt' mangoes. Starch content, titratable acidity, total sugars and flesh firmness evaluations also were done as described in Chapter 4.

ACC content of the mango mesocarp tissue was measured at the time of transfer to 20°C, 1 day after transfer and at the end of the transfer period. ACC extraction and determinations were done using the method of Lizada and Yang (1979) as modified by Hoffman and Yang (1980), but using 80% methanol (v/v) (Nieder *et al.*, 1986) as extractant and with no prior purification by ion exchange resin.

Electrolyte leakage (EL) of 'Tommy Atkins' mangoes was determined only at transfer to 20°C, while EL of 'Keitt' mangoes was measured at transfer and after 3 additional days using a YSI conductance bridge (Yellow Springs, OH), model 31A. From each replicate, 10, 8x5 mm mesocarp discs were excised with a cork borer from the median portion of the mesocarp and incubated for 3 hours at room temperature in 10 ml of a 0.4 M mannitol solution. EL was expressed as percent of total leachate after freezing/thawing.

The experiments were conducted in completely randomized design. Statistical analysis of the data was done using SAS for PC (SAS Institute Inc., Cary, NC).

Results and Discussion

Respiration rates of 'Tommy Atkins' and 'Keitt' mangoes stored at the different temperatures had patterns similar to those reported by Burg and Burg (1962), McCollum *et al.* (1993) and Yahia and Hernandez (1993) for low temperature storage of mangoes. The mangoes clearly responded to the reduced storage temperature with lower respiratory activity independent of ripeness stage. Comparing mature green to tree ripe 'Tommy Atkins' mangoes stored at 12°C (Figure 5.1), the mature green fruit had lower initial CO_2 production but, after 2 weeks in storage, the rates were similar. After transfer to air, the respiration rates of the mature green fruit stored at 10 or 25% CO_2 did not differ from the mature green and tree ripe control treatments, however the tree ripe fruit from CA had significantly lower CO_2 production rates. Gonzales *et al.* (1990) also observed reduced respiratory activity in 'Keitt' mangoes after transferring the fruit to air after storage for up to 2 weeks at 20°C in high density polyethylene bags in which O_2 concentrations had dropped to 10% and CO_2 concentrations had increased to about 5%.

Respiration rates of 'Tommy Atkins' mangoes stored at 8 or 12°C are presented in Figure 5.2. There was no difference in respiration rate between mature green and tree ripe mangoes stored at 12°C, but tree ripe fruit stored at 8°C had significantly lower respiratory activity. Veloz *et al.* (1977) observed the same reduction in respiration rates in 'Kent' mangoes with reduction of the storage temperature. Likewise, the authors reported a sharp increase in respiration, as seen with 'Tommy Atkins' (Figure 5.1), after transferring the fruit to 25°C. Veloz *et al.* (1977) concluded that this increase could not be considered the respiratory climacteric associated with ripening, but rather a consequence of suppressed metabolism by the low temperature during storage.

Ethylene production repeated the same pattern as respiratory activity (Figures 5.3 and 5.4). Ethylene production rates of control fruit in the first two experiments with 'Tommy Atkins' mangoes were very similar during storage at 8 or 12°C. The difference between the experiments was evident after transfer to air. Tree ripe 'Tommy Atkins' stored at 12°C continued to ripen in storage at 12°C and, as indicated by the deterioration rate at 20°C, senesced quickly after transfer to air. In contrast to the senescing processes of the tree ripe fruit at 12°C, the tree ripe 'Tommy Atkins' stored at 8°C did not ripen as fast because of the lower temperature and, therefore, were able to withstand the transfer period with greater quality retention. The sharp increases in respiration rates and ethylene production upon transfer to 20°C may reflect indicate tissue disorganization and deterioration.

Tree ripe 'Keitt' mangoes repeated the same patterns as the 'Tommy Atkins' mangoes. Respiration rates (Figure 5.5) and ethylene production (Figure 5.6) were much lower during the CA period than after transfer to air at 20°C, which probably was due to slower metabolic rates at the 5 and 8°C storage temperatures. The same intense increases after transfer to air at 20°C were also observed as with 'Tommy Atkins' mangoes stored at 12°C (Figures 5.1 and 5.2). The maximum levels of 50 ml CO_2/kg.hr^{-1} reached with 'Keitt' mangoes stored at 5 or 8°C were about half those of 'Tommy Atkins' stored at

12°C, which is in constrast to the report of Veloz *et al.* (1977), who determined that 'Kent' mangoes stored at 8°C reached the same respiratory rates as fruit stored at 13°C when measured 3 days after transfer to air at 25°C. Respiratory activity in 'Keitt' might have increased to reach rates similar to those observed in 'Tommy Atkins' and reported by Veloz *et al.* (1977) and Gonzales *et al.* (1990) if storage in air at 20°C had been continued beyond 3 days.

The onset of ethanol production by 'Tommy Atkins' mangoes stored at 12°C (Figure 5.7) advanced with increasing CO_2 concentrations in the storage atmosphere. Ethanol production by both mature green and tree ripe mangoes in the 45% CO_2 treatment increased continuously from the beginning of storage. Tree ripe fruit at 10 or 25% CO_2 started to produce significantly higher ethanol levels after 2 weeks of storage while ethanol production rates by mature green fruit in these atmospheres were low and continued low after transfer to air. Mature green and tree ripe controls had ethanol production rates during ripening at 20°C that were as high as either tree ripe or mature green fruit stored in 45% CO_2.

In the second experiment, mature green 'Tommy Atkins' fruit had higher ethanol levels after transfer to air, but still not as high as tree ripe fruit (Figure 5.8). Tree ripe mangoes stored in 25% CO_2 at 12°C had lower ethanol production than the mangoes of the same treatment in the previous experiment. This discrepancy in ethanol production between the two experiments may be explained by differences in ripeness stage, which, however, is not supported by color data as shown further in the discussion.

The mangoes from the 25% CO_2 treatment at 8°C had higher ethanol production compared to the fruit from the same treatment at 12°C. Higher ethanol under these conditions could derive from less terminal oxidation and slower flow of the TCA cycle, thus accelerating glycolysis for energy supply (Zemlianukhin and Ivanov, 1978).

The higher ethanol production rates in tree ripe compared to mature green mangoes probably derives from the higher energy requirements in the mature green fruit for ripening processes. Higher energy demand maintains electron flow through the oxidative phosphorylation pathway and, concomitantly, the TCA cycle, maintaining the carbon flow unhindered to support the required energy level and, therefore, avoiding build up of pyruvate. High energy demand translates into less reducing power availability. A high ratio of reduced to oxidized NAD^+ together with high pyruvate level are some of the driving forces for ethanol production (Ke *et al.*, 1993).

Ethanol production by 'Keitt' mangoes during the CA period (Figure 5.9A) was much lower than in 'Tommy Atkins'. The lower ethanol production rates of these tree ripe fruit, in contrast to the tree ripe 'Tommy Atkins', may reflect low energy demand associated with overall lower metabolic rate, reducing the possibility of pyruvate accumulation, which could enhance ethanol production. On the other hand, the 25% CO_2 treatment at both temperatures had significantly higher ethanol than the control or 10% CO_2 treatments during the storage period and after transfer to air (Figure 5.8 and 5.9B), which could reflect the effects of CO_2 on enzymes of the TCA cycle, mainly succinate dehydrogenase, as referred to by Monning (1983). If the carbon flow through the TCA cycle is impeded, then glycolysis is the only source of energy (Zemlianukhin and Ivanov, 1978). The drop in ethanol production after transfer of the mangoes to air in the 25% CO_2 treatment at 5°C may be explained by the high energy demand for repair, as discussed below, while at 8°C these repair mechanisms, if in place at all, were less intense.

Another possible mechanism for the increase in ethanol production might be related to lower O_2 concentrations in the innermost mesocarp layer of CA-stored fruit and fruit ripening at 20°C. Esguerra *et al.* (1990) reported a decrease in O_2 concentrations from 17 to 7% in vapor heat-treated mangoes in which respiration rates had increased 4 to 6 fold. These authors suggested that O_2 diffusion into mesocarp tissue at ambient temperatures could be limited by the well defined cuticle and small intercellular spaces at the epidermal layers in mango fruit. Mitcham and McDonald (1993) likewise reasoned that increased ethanol production in vapor heat-treated 'Tommy Atkins' and 'Keitt' mangoes could be ascribed to lower O_2 concentrations in the center of the fruit.

Storage of tree ripe mangoes in CA at 12°C for 3 weeks did not prove to be a viable alternative to ameliorate quality losses. In both experiments with 'Tommy Atkins' mangoes, ripening proceeded to such an extent that the fruit were not capable of enduring even a few more days in air at 20°C. Even though 10 or 25% CO_2 combined with 5% O_2 seemed to slow the ripening processes during the CA period compared to control fruit, especially when examining starch hydrolysis (Table 5.1), the performance of the fruit after transfer from these treatments to air was as poor as that of the control fruit. Because of high decay incidence (data not shown) no evaluations were performed after 5 days in air at 20°C on the mature green or tree ripe fruit stored at 12°C.

Tree ripe fruit stored at 8°C for 3 weeks, on the other hand, were much better in terms of visual quality. Ripening processes at 8°C were less intense, as indicated by lower respiratory activity (Figure 5.2) and significantly higher starch content, which allowed the fruit to withstand the subsequent 5 days at 20°C with less quality losses than fruit of the same ripeness stage stored at 12°C. There were no significant differences in titratable

Table 5.1: Quality parameters of 'Tommy Atkins' mangoes stored in air or CA for 21 days at 8 or 12°C plus 5 days in air at 20°C.

	Starch (% F.W.)		Total Sugars (mg/g F.W.)		Titratable acidity (% citric acid)		Firmness (Newton)	
	At transfer	+5 days/air	At transfer	+5 days/air	At transfer	+5 days/air	At transfer	+5 days/air
Mature green - 12°C								
Air	0.030	nd/[1]	94.65	nd	1.93	nd	21.58	nd
10%CO_2	0.073	nd	105.15	nd	1.31	nd	13.53	nd
25%CO_2	0.079	nd	78.52	nd	1.82	nd	28.44	nd
Tree ripe - 12°C								
Air	0.047	nd	100.90	nd	0.61	nd	4.90	nd
10%CO_2	0.075	nd	109.95	nd	1.06	nd	10.59	nd
25%CO_2	0.076	nd	127.48	nd	0.81	nd	7.06	nd
Tree ripe - 8°C								
Air	0.116	0.099	107.10	131.63	1.14	1.20	12.45	11.47
10%CO_2	0.318	0.077	106.00	111.03	0.92	1.03	14.71	13.14
25%CO_2	0.309	0.077	85.25	97.82	1.04	1.08	37.75	19.32
LSD ($p<.05$)	0.223/[2]		19.79		0.32		2.35	

/[1] nd=not determined.
/[2] overall LSD $p<.05$ for at transfer and +5 days at 20°C.

acidity between treatments and days of storage for tree ripe fruit stored at 8°C. Tree ripe control fruit stored at 12°C had significantly lower acidity than all the other treatments except tree ripe fruit in 25% CO_2 at 12°C. Mature green fruit stored at 12°C had the highest acid levels. Flesh firmness was the highest in the 25% CO_2 atmosphere for mature green mangoes stored at 12°C and tree ripe stored at 8°C. Firmness after transfer to air was retained only in fruits stored at 8°C.

Mangoes from the treatments that had the best performance in firmness also had the lowest total sugars levels compared to control fruit and the 10% CO_2 treatment. Starch contents did not differ statistically, though it is evident that CO_2 contributed to reduced starch breakdown during storage, which poses the question of the fate of this amount of carbohydrate. Probably ethanol production, as seen in Figure 5.8, is the major route of carbon losses. Tree ripe mangoes at 12°C, on the contrary, did not show significantly increased ethanol production during the CA period in this experiment (Figure 5.8B) and, probably because of enhanced respiratory activity of control fruit towards senescence (Figure 5.1), sugar levels were lower compared to the 25% CO_2 treatment.

Storage of mature green fruit at 12°C for 21 days did not show a significant improvement over tree ripe fruit in terms of quality. These fruit had the same intensity of respiratory activity as the tree ripe mangoes at 12°C (Figure 5.1). Mature green fruit also deteriorated quickly after transfer to air.

Another good indication that storage of tree ripe fruit at 12°C did not prevent ripening from advancing, becomes evident when examining electrolyte leakage at transfer to air after 21 days in CA (Figure 5.10). Mangoes stored in 25% CO_2 had lower EL than control fruit. At 8°C, the tree ripe fruit in both the 10% and 25% CO_2 atmospheres had reduced ion efflux compared to the air control.

Cote *et al.* (1993) concluded that electrolyte leakage is not always a reliable measure of chilling injury and membrane integrity, even though, in the present experiments with 'Tommy Atkins' mangoes the absence of elevated ion efflux at 8°C seems to indicate that no chilling injury was inflicted by storing tree ripe mangoes at 8°C. The higher levels of ion efflux of the tree ripe mangoes at 12°C are probably an indication of advanced ripeness as indicated by Fuchs *et al.* (1989).

Some of the work dealing with storage of mangoes at chilling temperatures, such as Chaplin *et al.* (1986), Thomas and Joshi (1988), Fuchs *et al.* (1989) and Chaplin *et al.* (1991), shows that the appearance of symptoms of chilling injury occurs in a few days after the exposure to the chilling temperature. This observation further corroborates the view that, at 8°C, tree ripe 'Tommy Atkins' mangoes, in which there were no visual symptoms of injury at transfer from 21 days in CA and after 5 days in air, were not injured and that the higher ion efflux noted in fruit stored at 12°C represented advanced ripeness rather than chilling injury.

Because of the poor performance of the tree ripe mangoes stored at 12°C and the improved quality at 8°C, and considering that 8°C did not cause chilling injury, a lower temperature, *i.e.*, 5°C, was also tested to determine if chilling injury would occur at the lower temperature. Mature green fruit were not stored at 8 or 5°C based on the conclusions of Chaplin *et al.* (1991) that ripe fruit are less chilling sensitive than mature green mangoes.

Quality parameters of 'Keitt' mangoes stored at 5 or 8°C are presented in Table 5.2. It is evident that the 'Keitt' mangoes from the 5°C CA storage could have been stored in air at 20°C for a few more days, which probably would have enhanced starch breakdown without negatively affecting parameters such as total sugars and titratable acidity. There was a residual effect on starch hydrolysis after transfer to air in the fruit stored in 10 or 25% CO_2 at 5°C and 25% CO_2 at 8°C, however, after transfer to 20°C, total sugars reached levels equal or nearly equal to the control fruits in all but fruit from the 25% CO_2 treatment at 5°C.

These high levels of starch present at the end of ripening, especially in the 25% CO_2 treatment in both temperatures and in the 10% CO_2 treatment at 5°C, might also indicate that starch hydrolases were somewhat affected by the presence of high levels of CO_2 and the low temperature. Chhatpar *et al.* (1971) have already indicated that chilling

Table 5.2: Quality parameters of 'Keitt' mangoes stored for 21 days in CA or air at 5 or 8°C plus 3 days in air at 20°C.

	Starch (% F.W.)		Total sugars (mg/g F. W.)		Titratable acidity (% citric acid)		Firmness (Newton)	
	At transfer	+5 days/air	At transfer	+5 days/air	At transfer	+5 days/air	At transfer	+5 days/air
5°C								
Air	0.62	0.37	75.29	88.46	1.48	1.33	94.44	14.61
10% CO_2	0.71	0.58	54.75	87.52	1.14	1.11	111.21	30.40
25% CO_2	0.66	0.67	49.52	58.50	1.58	1.09	90.22	63.74
8°C								
Air	0.38	0.28	88.45	95.15	1.56	1.36	18.04	14.80
10% CO_2	0.64	0.24	65.80	94.04	1.32	1.15	94.05	9.62
25% CO_2	0.62	0.60	75.25	86.69	1.11	0.94	107.88	13.14
LSD $p<.05$	0.12		5.91		0.13		26.98	

injury reduces starch breakdown in mangoes. Medlicott *et al.* (1990) observed the occurrence of a 'corky' layer underneath the peel of immature and mature green mangoes stored for 21 days at 8 or 10°C, which could be associated with reduced starch breakdown.

Higher levels of titratable acidity have been associated with chilling injury by Chaplin *et al.* (1991), while Fuchs *et al.* (1989) and McCollum *et al.* (1993) on the other hand, did not determine differences in titratable acidity in 'Haden' mangoes attributable to chilling temperatures. In the present experiments, tree ripe 'Keitt' and 'Tommy Atkins' stored at 5°C showed no indication of elevated titratable acidity. In fact, the fruit stored in CA had reduced titratable acidity levels after transfer to air at 20°C. The lower titratable acidity levels in CA-stored fruit after 3 days at 20°C were probably the result of acids being mobilized as respiratory substrate, since less sugars were available due to the suppression of starch hydrolysis.

Despite the fact that titratable acidity levels did not indicate that chilling injury occurred, there were some other indications that the 25% CO_2 treatment at 5°C did inflict damage on 'Keitt' mangoes. The first indication relates to the high flesh firmness in the 25% CO_2 treatment after 3 days in air at 20°C. This extremely high firmness value is in contrast to the observations of Chaplin *et al.* (1991) and Seymour and Tucker (1991), who have pointed out that softening is very difficult to prevent once ripening processes have started. Another indication that damage occurred in the 25% CO_2 treatment at 5°C can be determined by comparing ACC concentrations in 'Keitt' mangoes (Figure 5.11) with 'Tommy Atkins' mangoes (Figure 5.12) that had been stored for 14 days in the same conditions as the 'Keitt' mangoes. Levels of ACC were highest in the 25% CO_2 treatment at 5°C and continued to increase after transfer to air, while ACC levels in the other treatments did not differ significantly. There was no stimulation of ACC levels by the 25% CO_2 treatment at 8°C.

Cabrera and Saltveit (1990) observed accumulation of ACC in cucumbers after prolonged chilling, and speculated that the activity of ACC oxidase could be limited under chilling, therefore resulting in inhibition of ethylene production in the chilled cucumbers. Wang (1982), however, had already determined that chilling temperatures stimulate ethylene production in different species, and Chan *et al.* (1985), as well as McCollum and McDonald (1991), both considered determination of ethylene production to be a useful method to determine the onset of chilling injury in papayas and citrus, respectively. With 'Keitt' mangoes stored in 25% CO_2 at 5°C the ethylene production by CO_2 injured fruit did not differ from control fruit during storage at 5°C or after transfer to air at 20°C (Figure 5.6). However, while the control fruit did not have any visual indication of chilling injury, the ethylene production of both the control and 25% CO_2 treatments was threefold higher than that of the 10% CO_2 treatment after transfer to air at 20°C.

Chan *et al.* (1982) concluded that chilling temperatures did not have any deleterious effect on ACC oxidase in papaya fruit. ACC oxidase activity of 'Keitt' (Figure 5.13) and 'Tommy Atkins' (Figure 5.14) mangoes during CA storage concur with the observation of Chan *et al.* (1982) for papaya. The highest enzyme activity was detected in control fruit stored at 8°C, indicating that metabolism in air at 8°C proceeded normally, while the CO_2 treatments at the same temperature impeded ethylene evolution by significantly reducing the ACC oxidase activity. At 5°C, there was no additional effect of high CO_2 beyond that shown by the low storage temperature. After transfer to air from both temperatures, ACC oxidase activity increased to similar levels, except in control fruit stored at 8°C. The significantly higher ACC oxidase activity following 8°C storage in air probably reflects the more intense ripening processes. Therefore, the accumulation of ACC in 25% CO_2 at 5°C (Figures 5.11 and 5.12) may indicate that ACC synthase was up-regulated in the injured fruit. This observation is in agreement, at least in part, with Lelievre *et al.* (1995), who concluded that both ACC synthase and ACC oxidase are induced during chilling in pre-climacteric 'Granny Smith' apples and accumulate before any transfer to warmer temperature.

The results for EL of 'Keitt' mangoes (Figure 5.15) illustrate the difficulty inherent in interpreting data that are influenced both by tissue injury and tissue senescence (ripening). The reduced EL at transfer of the mangoes from the 10 and 25% CO_2 treatments at 8°C compared to the air control probably indicates that ripening was delayed during the CA storage period and, therefore, that the CA had a positive effect on cell and tissue integrity. After transferring the mangoes from the CA at 8°C to air at 20°C, however, the ripening processes resumed, causing ion efflux to also increase. The control fruit were at a more advanced ripeness stage at the time of transfer from 8 to 20°C, as indicated by their firmness levels (Table 5.2) and therefore showed no significant increase in EL. The CA treatments at 5°C also reduced EL compared to the air control. The control fruit at 5°C had EL levels equal to the control at 8°C at transfer, and also did not show an increase in EL during the 3 days at 20°C, however, the control fruit from 5°C were unripe at the time of transfer to 20°C (Table 5.2). Upon transfer from 5 to 20°C, the 25% CO_2 had significantly higher levels of EL than the fruit from 10% CO_2, and the EL did not increase during the subsequent 3 days at 20°C, in contrast to the 10% CO_2 fruit, which reached levels of EL equal to the (ripe) control fruit. It is possible that the elevated EL of control fruit at 5°C reflects chilling injury in that treatment, while the less dramatically elevated EL levels in the 25% CO_2 treatment at 5°C might reflect the CO_2 injury that was indicated by the scald-like symptoms on the epidermis (see below). The increases in EL that were seen after transfer of fruit from the CA treatments at 8°C, and the 10% CO_2 treatment at 5°C, to air at 20°C, probably reflect the loss of tissue integrity associated with ripening, while the lack of a similar increase in the 25% CO_2 treatment from 5°C corresponds to the inhibition of ripening seen in those fruit (Table 5.2).

'Tommy Atkins' mangoes stored for 14 days at 5 or 8°C, however, did not produce the same results as 'Keitt' mangoes in terms of EL (Figure 5.16). 'Tommy Atkins' fruit stored in 10 or 25% CO_2 at 5°C had significantly higher EL than control fruit. These higher values of ion efflux in the CA treatments may be an indication that the main cause of increased efflux, as observed in both cultivars, is CO_2 injury that was enhanced at the lower temperature rather than chilling injury. The low EL level in the control at 5°C suggests that chilling injury did not occur in the 'Tommy Atkins' fruit, either due to the shorter exposure time at 5°C or, perhaps, because the maturity of the 'Tommy Atkins' fruit was more advanced than in the 'Keitt'. The progressive decrease in EL with increasing CO_2 levels at 8°C probably reflects inhibition of ripening by high CO_2 treatments.

Visual symptoms, *i.e.*, scald-like dark patches, were observed only on the mangoes from the 25% CO_2 treatment at 5°C, which probably derive from degradation of chlorophylls to pheophytins, as discussed in Chapter 3. There was neither an enhancement nor an alleviation of the symptoms after transfer to air at 20°C. Moreover, the symptoms were confined to the epidermis, there was no distinct pattern of distribution, and no sunken lesions were associated with the dark patches.

Ground color development has also been shown to be affected when mangoes are damaged by chilling injury. In examining the epidermal and mesocarp tissue color of 'Keitt' mangoes (Table 5.3), however, it is evident that only the 25% CO_2 treatment at 5°C affected epidermal chroma values compared to the control and 10% CO_2 treatments, both at transfer and after the mangoes had been in air at 20°C for 3 days. The relatively small changes in hue angles during storage at 5°C compared to the significant decreases at 8°C indicate that temperature alone was very effective in slowing color changes. Control fruit did not change in hue angle after transfer to 20°C, maintaining significantly higher values after 3 days than fruit from the 10 and 25% CO_2 treatments. The lower hue angles in fruit from the CA treatments at 8°C may have been due to higher endogenous ethylene

Table 5.3: Epidermal and mesocarp hue and chroma values of 'Keitt' mangoes stored in CA or air for 21 days at 5 or 8°C plus 3 days in air at 20°C.

	Epidermal color				Mesocarp color			
	Hue		Chroma		Hue		Chroma	
	At transfer	+3 days	At transfer	+3 days	At transfer	+3 days	At transfer	+3 days
5°C								
Initial	103.36		29.04		95.20		58.05	
Air	103.18	103.67	32.33	35.10	91.22	93.11	60.21	59.14
10% CO_2	103.20	95.63	33.71	31.00	95.27	95.51	54.66	53.09
25% CO_2	102.55	97.45	28.76	26.97	97.16	96.68	54.42	51.47
8°C								
Initial	109.75		29.39		95.20		58.05	
Air	101.93	103.98	34.12	34.13	92.61	94.02	58.84	56.20
10% CO_2	103.13	99.84	34.44	36.52	95.40	91.40	51.82	60.42
25% CO_2	101.86	98.44	32.96	32.99	94.24	91.39	56.00	61.82
LSD $p<.05$	3.54		2.28		1.16		3.36	

levels in those fruit after transfer to air at 20°C compared to the 8°C air control (Figure 5.6). With the fruit stored at 5°C, epidermal color was not related to ethylene production, since the chill-injured control fruit and CO_2-injured 25% CO_2 treatment fruit had much higher ethylene levels than the 10% CO_2 fruit (Figure 5.6), but hue angle was lowest in the 10% CO_2 treatment after 3 days in air at 20°C.

CA fruit also had higher (greener) mesocarp hue values than the controls at transfer in both storage temperatures (Table 5.3). Mesocarp hue angle values subsequently significantly increased in control fruit during the 3 days in air at 20°C, but stayed the same in CA-stored fruit from 5°C and decreased in CA-stored fruit from 8°C. The color of mesocarp tissue of chilled mango fruit has been reported as being pale (Chaplin *et al.*, 1986; 1991), which has been associated by Thomas and Joshi (1988) with reduced carotenoid synthesis in the mesocarp. In the present experiments with 'Keitt' mangoes, it seemed that a more pale yellow mesocarp color, as indicated by lower chroma, was more influenced by CO_2 atmospheres than by the storage temperature. This suggests that this supposed chilling injury symptom is a consequence of inhibition of normal ripening-related color development. Significantly higher ethylene production after transfer from 8°C to air at 20°C (Figure 5.6) could have enhanced ripening and the associated pigment changes in the mesocarp as seen with 10 and 25% CO_2 mangoes.

The epidermal color of mature green and tree ripe mangoes stored at 12°C (Tables 5.4 and 5.5) was consistent with color determinations in previous experiments, except for the 45% CO_2 treatment. Mangoes from this treatment had significantly lower chroma values after storage compared to the control and 10 and 25% CO_2 treatments, which is a sure indication of damage by CO_2, enhancing browning due to polyphenolic oxidation as indicated by Lakshminarayana (1980).

Table 5.4 Epidermal hue and chroma values of mature green and tree ripe 'Tommy Atkins' mangoes stored for 21 days in air or CA at 12°C plus 5 days in air at 20°C.

	Hue		Chroma	
	At transfer	+5 days in air	At transfer	+5 days in air
Mature green				
Initial	107.96		29.78	
Air	92.71	75.40	37.37	42.64
10% CO_2	104.30	84.45	32.35	39.79
25% CO_2	104.81	89.09	37.38	41.28
45% CO_2	90.38	nd /[1]	20.41	nd
Tree ripe				
Initial	83.68		39.36	
Air	72.10	48.29	44.91	48.29
10% CO_2	78.23	56.77	43.25	56.77
25% CO_2	74.45	63.56	40.78	63.56
45% CO_2	71.64	nd	33.14	nd
LSD $p<.05$	5.99		3.19	

/[1] nd=not determined.

Table 5.5 Epidermal hue and chroma values of mature green and tree ripe 'Tommy Atkins' mangoes stored in air or CA for 21 days at 8 or 12°C plus 5 days in air at 20°C.

	Hue		Chroma	
	At transfer	+5 days in air	At transfer	+5 days in air
Mat. Green/12°C				
Initial	106.73	nd/[1]	29.51	nd
Air	77.64	nd	31.61	nd
10% CO_2	96.18	nd	34.46	nd
25% CO_2	100.14	nd	34.55	nd
Tree ripe/ 12°C				
Initial	81.95	nd	31.82	nd
Air	65.69	nd	38.93	nd
10% CO_2	72.75	nd	39.32	nd
25% CO_2	73.48	nd	35.84	nd
Tree ripe/ 8°C				
Initial	67.39		32.81	
Air	69.97	60.39	39.15	40.03
10% CO_2	66.44	66.80	34.76	34.88
25% CO_2	64.21	63.99	36.87	37.03
LSD $p<.05$	6.11		6.10	

/[1] nd=not determined.

Another noteworthy ground color change occurred with the control tree ripe 'Tommy Atkins' at 12°C. The significantly lower values for chroma after 5 days in air at 20°C (Table 5.4), do not indicate that these fruit had less intense color changes than fruit stored in the CO_2 atmospheres, but rather that the different pigments respond differently to tissue disorganization in senescent fruit. Moreover, tissue disorganization may result in more intensive oxidation of pigments, resulting in browning as indicated by the low chroma.

Conclusions

Storage of tree ripe mangoes in CA at 12°C for 21 days was not a viable alternative to air storage of mature green fruit for the same time and at the same temperature due to excessive ripening during storage. Storage of tree ripe fruit at 8°C considerably increased the quality of mangoes stored for 21 days and these fruit did not have any indication that injury had been inflicted by storage at a lower than recommended temperature. Tree ripe mangoes stored in 10 or 25% CO_2 at 8°C had the best quality after 21 days plus 3 or 5 days in air at 20°C. Tree ripe mangoes stored in 25% CO_2 at 5°C showed evidence of CO_2 injury, while elevated electrolyte leakage and ethylene production in the air control 'Keitt' fruit suggested that slight chilling injury may have occurred, while no increase in electrolyte leakage occurred in 'Tommy Atkins' at 5°C. The best quality in 5°C storage was maintained by the 10% CO_2 treatment.

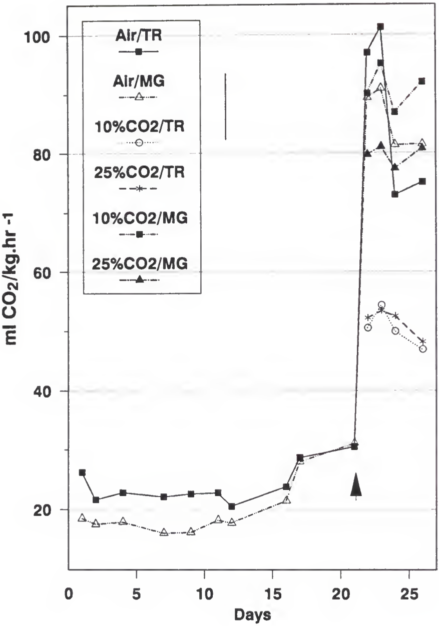

Figure 5.1: Respiration rates of mature green (MG) and tree ripe (TR) 'Tommy Atkins' mangoes stored for 21 days in CA or air at 12°C plus 5 days in air at 20°C. Arrow indicates transfer to air. (Vertical bar = LSD p<.05).

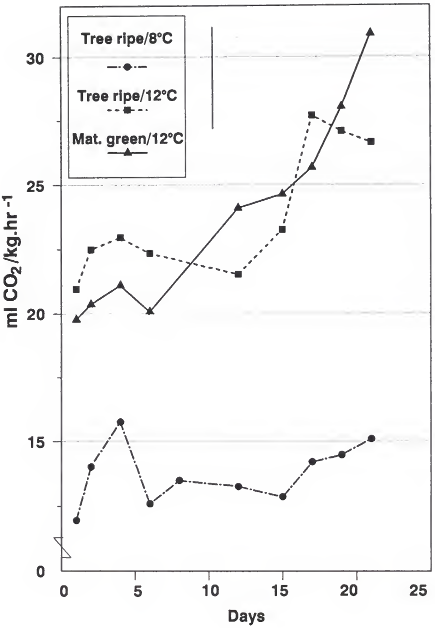

Figure 5.2: Respiration rates of mature green and tree ripe 'Tommy Atkins' mangoes stored for 21 days in air at 8 or 12°C. (Vertical bar = LSD p<.05).

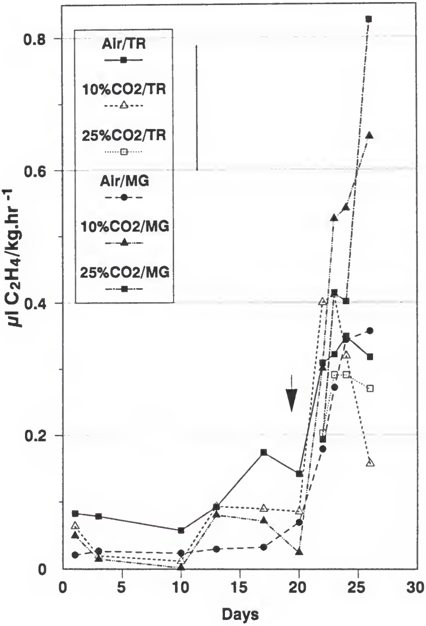

Figure 5.3: Ethylene production of mature green (MG) and tree ripe (TR) 'Tommy Atkins' mangoes stored for 21 days in CA or air at 12°C plus 5 days in air at 20°C. Arrow indicates transfer to air. (Vertical bar = LSD p<.05).

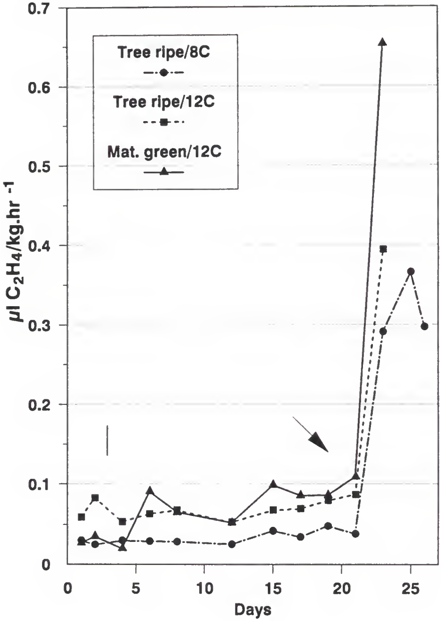

Figure 5.4: Ethylene production of mature green and tree ripe 'Tommy Atkins' mangoes stored for 21 days in air at 8 or 12°C plus 5 days in air at 20°C. Arrow indicates transfer to air. (Vertical bar = LSD p<.05).

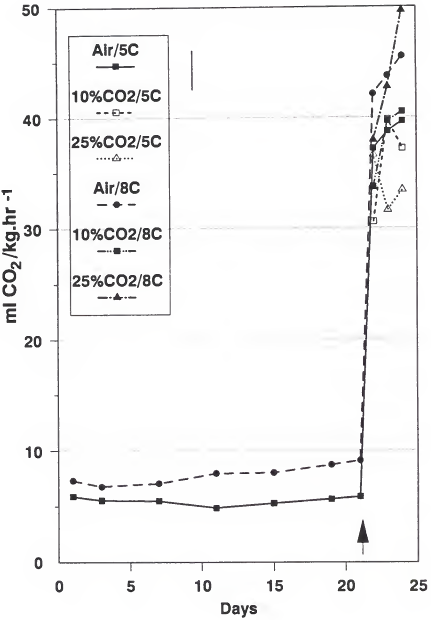

Figure 5.5: Respiration rates of tree ripe 'Keitt' mangoes stored for 21 days in CA or air at 5 or 8°C plus 3 days in air at 20°C. Arrow indicates transfer to air. (Vertical bar = LSD p<.05).

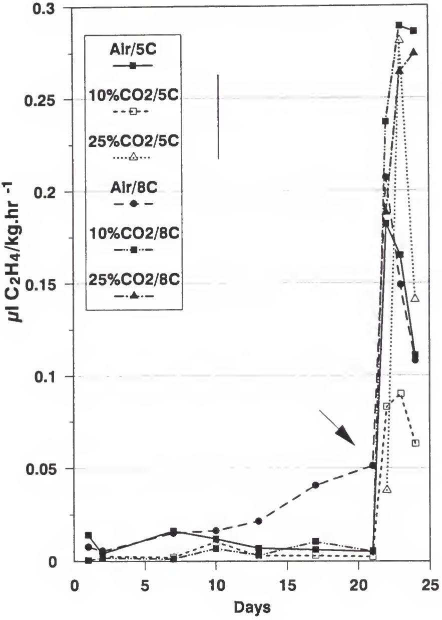

Figure 5.6: Ethylene production of tree ripe 'Keitt' mangoes stored for 21 days in CA or air at 5 or 8°C plus 3 days in air at 20°C. Arrow indicates transfer to air. (Vertical bar = LSD p<.05).

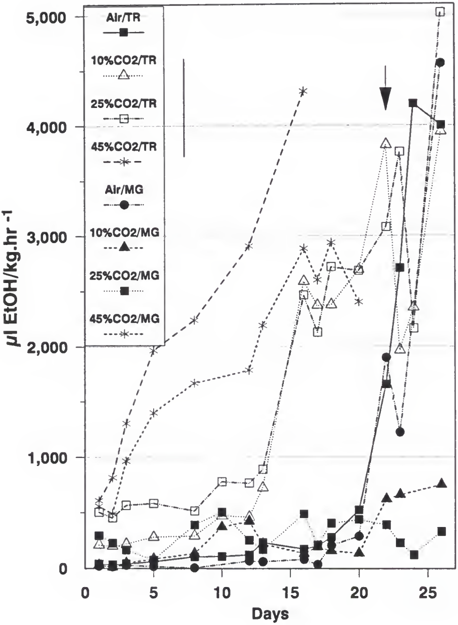

Figure 5.7: Ethanol production of mature green (MG) and tree ripe (TR) 'Tommy Atkins' mangoes stored for 21 days in CA or air at 12°C plus 5 days in air at 20°C. Arrow indicates transfer to air. (Vertical bar = LSD p<.05).

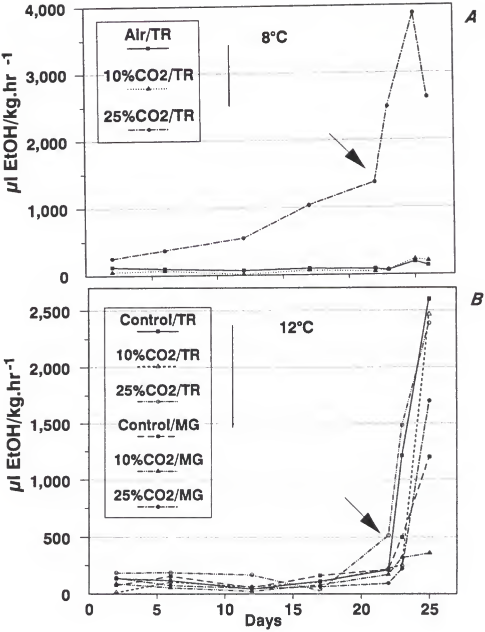

Figure 5.8: Ethanol production of mature green (MG) and tree ripe (TR) 'Tommy Atkins' mangoes stored for 21 days in CA or air at 8 (*A*) or 12°C (*B*) plus 5 days in air at 20°C. Arrow indicates transfer to air. (Vertical bar = LSD p<.05).

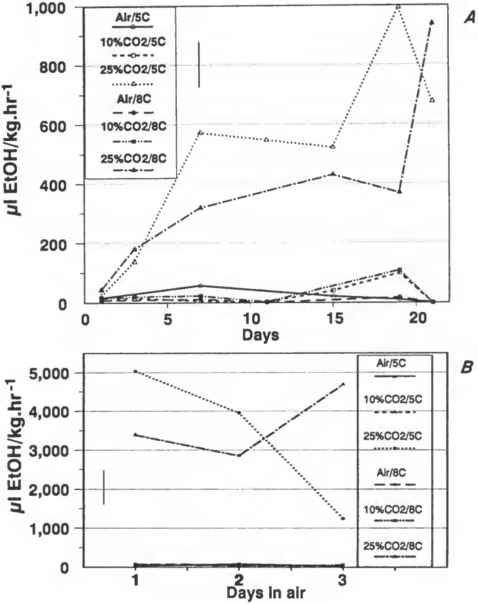

Figure 5.9: Ethanol production of tree ripe 'Keitt' mangoes stored for 21 days in CA or air at 5 or 8°C (*A*) plus 3 days in air at 20°C (*B*). (Vertical bar = LSD $p<.05$).

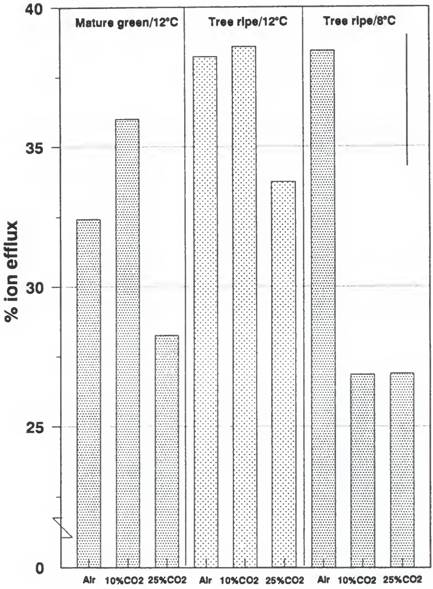

Figure 5.10: Ion efflux of mature green and tree ripe 'Tommy Atkins' mangoes stored for 21 days in CA or air at 8 or 12°C. (Vertical bar = LSD p<.05).

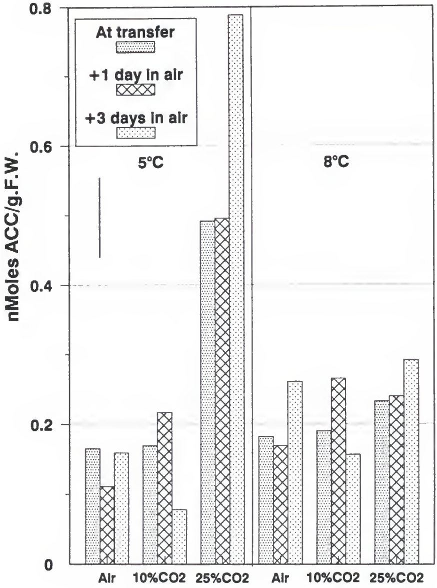

Figure 5.11: ACC concentrations in mesocarp tissue of tree ripe 'Keitt' mangoes stored for 21 days in CA or air at 5 or 8°C plus 3 days in air at 20°C. (Vertical bar = LSD p<.05).

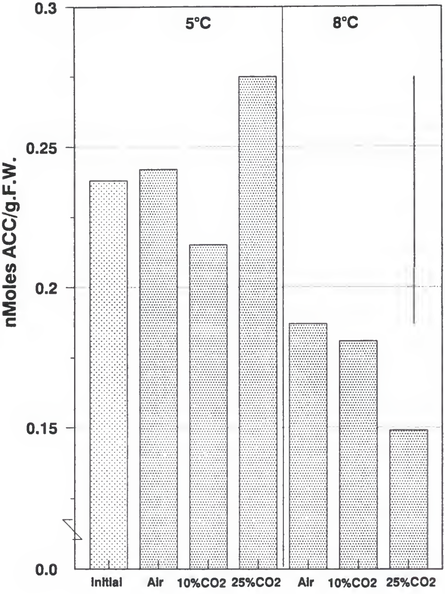

Figure 5.12: ACC concentrations in mesocarp tissue of tree ripe 'Tommy Atkins' mangoes stored for 14 days in CA or air at 5 or 8°C. (Vertical bar = LSD p<.05).

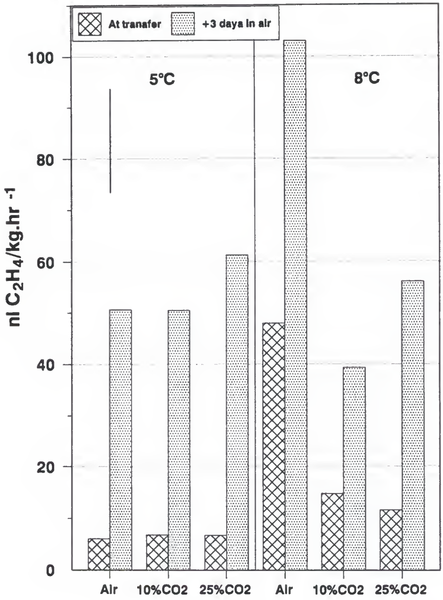

Figure 5.13: *In vitro* activity of ACC oxidase in mesocarp tissue of tree ripe 'Keitt' mangoes stored for 21 days in CA or air at 5 or 8°C plus 3 days in air at 20°C. (Vertical bar = LSD $p<.05$).

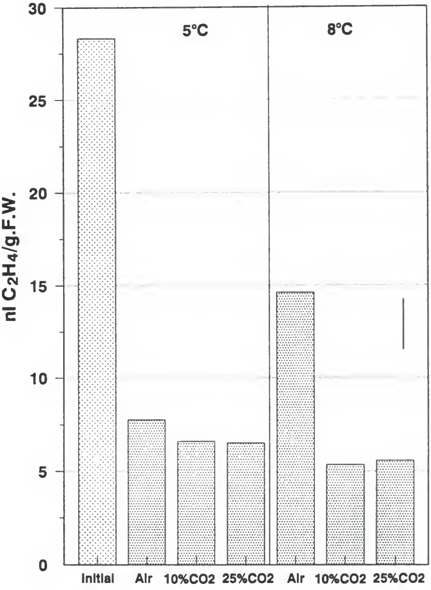

Figure 5.14: *In vitro* activity of ACC oxidase from mesocarp tissue of tree ripe 'Tommy Atkins' mangoes stored for 14 days in CA or air at 5 or 8°C. (Vertical bar = LSD $p<.05$).

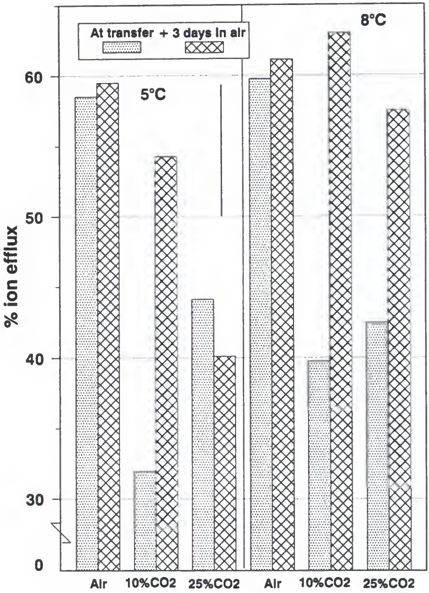

Figure 5.15: Electrolyte leakage of tree ripe 'Keitt' mangoes stored for 21 days in CA or air at 5 or 8°C plus 3 days in air at 20°C. (Vertical bar = LSD p<.05).

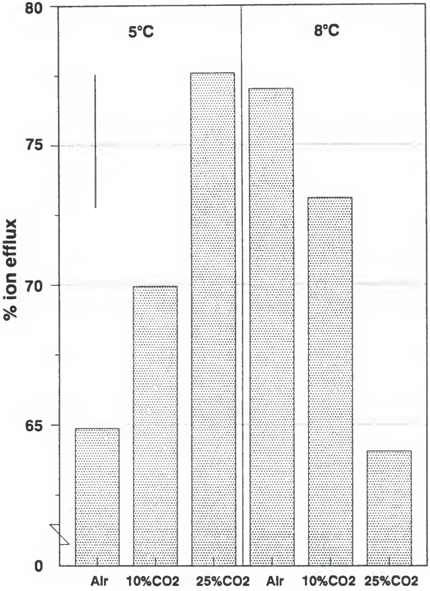

Figure 5.16: Electrolyte leakage of tree ripe 'Tommy Atkins' mangoes stored for 14 days in CA or air at 5 or 8°C. (Vertical bar = LSD p<.05).

CHAPTER 6
EFFECTS OF ELEVATED CO_2 ON ETHYLENE BIOSYNTHESIS

Introduction

The delaying effects of CO_2 on ripening processes have been attributed to its effects on ethylene biosynthesis. This conclusion is derived from observations of reduced ethylene production rates under elevated CO_2 atmospheres occurring in conjunction with delay in ripening processes, as for example, less chlorophyll breakdown and maintenance of higher flesh firmness (Kerbel *et al.,* 1988; Ke *et al.*, 1990) and reduced oxidation of organic acids (Yoshida *et al.*, 1986). These processes as well as starch breakdown (Hubbard *et al.*, 1990) have been shown to be responsive to ethylene (Matsumoto *et al.*, 1983; Tian *et al.*, 1994).

Bufler (1984) observed that ACC synthase activity in apple tissues was severely reduced in CO_2 atmospheres up to 10%, which led the author to conclude that lower ACC synthase activity could be responsible, in part, for the inhibition of ripening. However, it had been shown earlier by Kao and Yang (1982) that a low level of CO_2 is required for ethylene synthesis. More recently, Smith and John (1993) demonstrated that ACC oxidase has a requirement for CO_2 for its maximum activity, although there was an increase in the apparent K*m* for both ACC and O_2. Furthermore, it has been reported that CO_2 exerts its action on ethylene synthesis by activating the enzyme ACC oxidase rather than the substrate ACC (Fernandez-Maculet *et al.*, 1993).

In the previous chapters, it has been observed that ethylene production in mangoes rapidly dropped to undetectable levels after exposure to atmospheres of 25% CO_2 and above, in contrast to the observations of Rothan and Nicolas (1994) who found that CO_2 concentrations up to 28% only slightly reduced the ethylene production of kiwi fruit tissues. The inhibition of ethylene production in mango fruit by 25% CO_2 could not be attributed to tissue damage since ethylene production and other ripening processes recovered to control levels after transfer to air. Considering that the very rapid inhibition of ethylene production ($\leq$ 2 h) in response to the elevated CO_2 atmosphere probably rules out depletion of ACC as a mechanism for CO_2-induced inhibition of ethylene production and because of the requirement for CO_2 as an activator of ACC oxidase, the objective in the present experiments was to determine if the effects of elevated CO_2 atmospheres on ethylene synthesis could be attributed to the action of CO_2 upon ACC oxidase.

Material and Methods

Three long term storage experiments of 14 or 21 days duration were conducted. After the storage period, the mangoes were transferred to air at 20°C for 3 or 5 days to simulate commercial handling practices. Also, short term experiments, with durations of 2 to 6 hours, were conducted with intact fruit and mesocarp tissue plugs.

The first long term experiment was conducted for 21 days at 12°C plus 5 days in air at 20°C with 'Tommy Atkins' mangoes harvested from a grove of Brooks Tropicals in Homestead, Florida. The mangoes were transported by car to Gainesville the day after harvest. Upon arrival, the mangoes were hand graded for uniform size and ripeness stage and freedom from defects, and visually categorized as mature green or tree ripe based on ground color and fruit shape as described in Chapter 5. The hue angles determined by means of a chromameter at the beginning of the experiment for the epidermal ground color of mature green and tree ripe mangoes were: 107.96±1.90 and 83.68±6.54, respectively. Three replicates of four fruit per treatment and ripeness stage were placed in 10.05-l glass jars fitted with screw-top lids and inlet and outlet tubes. The treatments, 10, 25 or 45% CO_2 combined with 5% O_2 and an air control in a flow-through system, were applied to both ripeness stages as described in Chapter 5.

The second experiment was conducted for 21 days at 5 or 8°C plus 3 days in air at 20°C with tree ripe 'Keitt' mangoes harvested at Gulf Island Grove in Pine Island/Fort Myers, Florida. The fruit were transported by car to Gainesville the day after harvest. Upon arrival, the fruit were immersed in 53°C water containing 1000 ppm tiabendazole for 3 minutes, air dried and selected for freedom from defects and transferred to the storage rooms in 10.05-l glass jars. The treatments, a flow-through system of 10 or 25% CO_2 and an air control, were applied as described in Chapter 5.

The third experiment was conducted for 14 days at 5 or 8°C with 'Tommy Atkins' mangoes imported from Mexico by Brooks Tropicals in Homestead, Florida. These mangoes were handled under normal commercial conditions, treated with 46°C water for 60 minutes for insect disinfestation, and coated with a commercial formulation of carnauba wax prior to shipment to Florida by refrigerated truck. This experiment repeated the treatments of the second experiment, with 'Keitt' mangoes.

In the short term experiments, individual 'Tommy Atkins' mangoes from the same lot of the last (third) experiment were used. The mangoes were placed in 1.75-l glass jars at 8°C and exposed to 25% CO_2 plus 5% O_2 for 4 hours in a flow-through system. Control fruit were held in air in the flow-through system.

Besides individual fruits, mango mesocarp tissue was used to reduce variability between replicates, which is considerable among individual fruits. Ten cylindrical plugs, 8 x 5 mm, prepared from the median portion of mango mesocarp were exposed in 65-ml glass vials at 12°C to the same atmospheres as the individual fruits.

During the CO_2 treatments, 0.5-ml headspace gas samples were taken for ethylene measurements as described in the previous chapters. ACC concentrations were determined after CO_2 treatments using mesocarp tissue from individual fruits or from the tissue plugs exposed to CO_2. ACC concentrations were determined as described in Lizada and Yang (1979) and modified by Hoffman and Yang (1980), but using 80% methanol (v/v) as the extractant (Nieder *et al.*, 1986), and with no prior purification by ion exchange resin.

ACC oxidase activity *in vivo* was determined as described in Hoffman and Yang (1982) from mesocarp plugs vacuum infiltrated for 1 minute in a solution containing 1 mM ACC plus 2% KCl. ACC oxidase activity *in vitro* was determined as described in Fernandez-Maculet and Yang (1992) with slight modifications. The extraction buffer was 0.4 M 3-(N-morpholino)propane-sulfonic acid (MOPS) instead of TRIS (Tris[hydroxymethyl]aminomethane), and the assay buffer had the following composition: 1.64 ml of 50 mM MOPS (pH 7.2), 10% glycerol (v/v) and 20 mM sodium bicarbonate buffer, to which a 0.2-ml aliquot of crude ACC oxidase extract was added. Just prior to the 1 hour incubation at 30°C, 40µl of each of the following reagents were added to the test tube: 250 mM sodium ascorbate, 1 mM $FeSO_4 \cdot 7H_2O$, 50 mM dithiothreithol (DTT) and 50 mM ACC.

ACC synthase was extracted as described by Su *et al.* (1984) with the exception that the crude extract was dialyzed for 18 hours against 300 volumes of dialysis buffer. After dialysis, 400 µl of the extract was added to 60µl 600 mM N-[2-hydroxyethyl]piperazine-N'-[3 propanesulfonic acid] (EPPS), 0.1 mM DTT and 5 µM pyridoxal phosphate buffer at pH 8.5 plus 90µl of deionized water. The reaction was started by adding 60 µl of 0.5 mM S-adenosylmethionine. ACC concentrations were determined as described above after 3 hours of incubation at 30°C.

Mesocarp tissue from 'Tommy Atkins' mangoes stored for 14 days in CA, as described in Chapter 5, and 'Tommy Atkins' mangoes exposed for 4 hours to 25% CO_2 at 8°C was ground in liquid N_2 and prepared for western blotting. An equal amount (v/w) of 2x sample buffer at pH 6.8 containing 0.125 M Tris, 4% sodium dodecyl sulfate (SDS), 50% glycerol, 1.4 M β-mercaptoethanol, 2 mM phenylmethylsulfonyl fluoride (PMSF) and 0.05% bromophenol blue was added to the ground tissue powder. After boiling in a water bath for 5 minutes, the samples were frozen at -20°C until used for electrophoresis on SDS polyacrylamide gel. Aliquots of sample buffer containing protein from 0.50 g F.W. mesocarp tissue were loaded onto SDS gels. The gels were washed in transfer buffer (50 mM Tris, 40 mM glycine, 0.037% SDS and 20% methanol) and blotted onto a nitrocellulose membrane using a Bio-Rad semi-dry transfer apparatus (Bio-Rad Laboratories, Hercules, CA). After the transfer, the nitrocellulose membrane was incubated overnight at 4°C in 10% nonfat dried milk (Carnation brand) in TBST (20 mM Tris, pH 7.6; 137 mM NaCl; 0.1% Tween 20). The next day, after one 15-minute and

2x5-minute washes in TBST, the nitrocellulose was incubated for 1 hr at room temperature and constant agitation in goat anti-ACC oxidase antibodies obtained from Dr. H. Klee (prepared as described in Klee *et al.* (1991) and Klee (1993)) in 5% nonfat dried milk in TBST. Following the first incubation, the nitrocellulose was washed in TBST (3x15 minutes) and incubated for 45 minutes with rabbit anti-goat antibodies linked to horseradish peroxidase (Kirkegaard & Perry Laboratories, Gaithersburg, MD) in 50 ml of 5% nonfat dried milk in TBST. After the second incubation, the gels were washed 3x15 minutes in TBST and visualized with enhanced chemiluminescence (ECL) reagents according to the manufacturer's (Amersham Int., Little Chalfont, UK) instructions.

Statistical analysis of the data was done using SAS for PC (SAS Institute Inc., Cary, NC) in a split plot design having ripeness stage as the main factor.

Results and Discussion

Ethylene production by 'Tommy Atkins' mangoes stored for 14 days at 5 or 8°C is presented in Figure 6.1. Mangoes stored in the 25% CO_2 treatment at either temperature had no detectable ethylene during the 14 days of CA. The same effect was observed in all experiments as reported in the previous chapters. Only the control fruit at 8°C showed increased ethylene production during storage. The 10% CO_2 treatment at both temperatures reduced but did not completely suppress the ethylene production.

Examining the ACC concentrations and the activity of ACC oxidase (Figure 6.2) it could be concluded that the reduction in ethylene production in CA may be predominantly attributed to the effects of CO_2 on ACC oxidase. The mangoes stored in air at 8°C (Figure 6.2A) had significantly higher ACC oxidase activity compared to the other treatments, which indicates that at this temperature ethylene synthesis was not hindered. The ACC oxidase activity of the control mangoes at 8°C was comparable to the ethylene production rates of mangoes stored at 12°C, *e.g.*, tree ripe 'Haden' and 'Tommy Atkins' mangoes, whose ethylene production ranged from 0.04 to 0.15 µl C_2H_4/kg.hr^{-1}, as seen in Chapter 4.

The other treatments at 5 and 8°C did not differ in ACC oxidase levels, which was unexpected considering that in the 25% CO_2 treatment no ethylene was detected during the CA period. One consideration is that the presence of high CO_2 may be required during the extraction and measurement of ACC oxidase in order for the inhibition to be retained. As soon as the CO_2 concentrations are reduced, as would occur during extraction under ambient conditions, the enzyme could regain its activity.

The effects of CO_2 could also be ascribed to inhibition of ACC synthase since that enzyme is the rate limiting step in the ethylene biosynthetic pathway (Yu *et al.*, 1979). Moreover, Bufler (1984) has already determined that, in apples, CO_2 concentrations up to 10% were, at least in part, responsible for the reduced ethylene production via inhibition of the ACC synthase activity. But with mangoes it does not appear that the activity of ACC synthase is the major limiting factor, because ACC concentrations in CA did not differ from the controls (Figure 6.2B), even though ethylene production was low in 10% CO_2 and not detectable in the 25% CO_2 treatment (Figure 6.1). Examining the activity *in vitro* of ACC synthase (Figure 6.3) there is further indication that elevated CO_2 atmospheres reduce the activity of ACC oxidase leading to an accumulation of ACC. The significantly lower activity of ACC synthase in the 25% CO_2 treatment compared to control fruit at 8°C resulted in slightly lower ACC concentrations (Figure 6.2A), which is in accordance with the results of Bufler (1984). The results suggest that both ACC synthase and ACC oxidase were inhibited by 25% CO_2 at 8°C and, because of the presence of ACC in concentrations similar to control fruit at 8°C, but with no ethylene production, the predominant effect of the CO_2 atmosphere may be on ACC oxidase. The 10% CO_2 atmosphere did not completely suppress ethylene production (Figure 6.1), indicating that neither ACC synthase nor ACC oxidase of mangoes under this atmosphere were completely inhibited, which was confirmed by the results showing inhibition of ACC synthase equal to that of the 25% CO_2 treatment (Figure 6.2B) while ACC oxidase activity was twofold the level in the 25% CO_2 fruit (Figure 6.3).

Even though the activity of ACC synthase at 8°C decreased with increasing CO_2 concentrations in the storage atmosphere, the presence of ACC in the mango mesocarp tissues after 14 days in CA indicates that either ACC synthase was not completely inhibited or that ACC oxidase was inhibited during CA exposure but recovered during extraction. At 5°C, on the other hand, ACC synthase activity increased with increasing CO_2 concentrations (Figure 6.3). This increase is probably a stress response to CO_2 enhanced by the chilling temperature, and not an effect of CO_2 inducing *de novo* synthesis of ACC synthase as determined by Mathooko *et al.* (1995) in cucumbers treated with 60% CO_2 plus 20% O_2 at 25°C for 12 hours, leading to higher ACC concentrations and higher ethylene production compared to controls held in air at 25°C.

Lelievre *et al.* (1995) reported that both ACC oxidase and ACC synthase were present in higher amounts and much earlier in apples stored at 4°C compared to apples stored at 17°C. Ethylene production was lower in mangoes at 5°C than in mangoes stored at 8°C (Figure 6.1), while the activity of ACC oxidase was similar. This situation would be expected to indicate very similar ACC synthase activity at the two lower temperatures, but, on the contrary, there was higher ACC synthase activity in the mangoes in the 25% CO_2 atmosphere at 5°C, which, again, leads to the conclusion that the ACC accumulation was predominantly derived from the effects of the CO_2 treatment on ACC oxidase activity as has been observed by Cheverry *et al.* (1988).

Both mature green and tree ripe fruit stored at 12°C showed decreasing *in vivo* activity of ACC oxidase with increasing CO_2 concentrations (Figure 6.4A), causing ACC to accumulate (Figure 6.4B). The effects of CO_2 on ACC oxidase activity during storage seemed to be more pronounced in tree ripe fruit than in mature green fruit (Figure 6.4A). The accumulation of ACC after 21 days of CA storage to levels of about 0.25 nMoles ACC/g·F.W. for mature green fruit and between 0.6 to 0.9 nMoles/g·F.W. in the tree ripe mangoes indicates that ACC synthase activity was neither significantly enhanced by CO_2 atmospheres as reported by Mathooko *et al.* (1995) in cucumbers, nor was it inhibited as observed by Bufler (1984) and Levin *et al.* (1993) in apples, but rather reflected the differences in ripeness stage of the mangoes. Mature green fruit had lower ACC concentrations than the tree ripe fruit because the former were at a less advanced ripeness stage.

After transfer from CA at 12°C to air at 20°C, the mature green and tree ripe fruit had significant increases in ACC oxidase activity (Figure 6.4A) and ACC concentrations (Figure 6.4B) because of enhanced ripening at the higher temperature. The air control fruit had lower ACC oxidase activity and higher ACC levels 5 days after transfer to 20°C, reflecting their overripe state. The 45% CO_2-treated fruit were not analyzed after transfer because of CO_2 damage as discussed in previous chapters. It is noteworthy, however, to examine the different responses in activity of ACC oxidase to 10 and 25% CO_2 at 12°C (Figure 6.4B). With tree ripe fruit, increasing CO_2 concentrations limited ripening processes to a greater extent than in mature green fruit, as seen in Chapter 5 for color development and other quality parameters, so that lower ACC oxidase activity might also reflect some inhibition of ripening in the already initiated fruit. On the other hand, mature green mangoes stored at 25% CO_2 seemed to be under more stress, as indicated by a twofold higher ethanol production in this treatment (see Chapter 5), compared to the mangoes at 10% CO_2, which resulted in increased enzyme activity. Therefore, this enhanced activity of ACC oxidase may reflect a stress response to the extreme CO_2 environment.

Neither immersion of mangoes in 46°C water for 60 minutes for insect disinfestation or immersion in 53°C water for 3 minutes for postharvest anthracnose control significantly affected ACC oxidase activity. In a preliminary experiment (data not shown), the *in vivo* activity of ACC oxidase of 'Keitt' mangoes from either hot water treatment had returned to the initial activity levels after 4 days of storage at 12°C. Significantly enhanced levels of ACC oxidase were found only immediately after the fruit had equilibrated to room temperature following the hot water treatments, an effect that was more pronounced in the outer layers of the mesocarp tissue. Thus, it does not appear likely that the hot water treatments received by the imported mangoes influenced their response to CA treatments.

If the half-life of mango ACC oxidase is in the range of 4 to 5 hours, as estimated by Cheverry *et al.* (1988) for avocados, and the complete inhibition of ethylene in 25% CO_2 production is due only to inhibition of ACC oxidase, then no enzyme should be detected after 2 weeks of storage, which was not the case in these experiments (Figures 6.2A and 6.4A). The recovery of ethylene production in mango mesocarp tissue that did not produce any ethylene after a 60 minute exposure to 25% CO_2 was almost immediate upon return to air (Table 6.1), while tissue that was in air and then transferred to 25% CO_2 showed a significant drop in ethylene production after only 30 minutes of exposure to the elevated CO_2 level. A similar decrease in ethylene production was observed by Chaves and Tomas (1984) with apples in a flow-through system of 20% CO_2 at 20°C for 2 hours, which was attributed by the authors to the effects of CO_2 on ACC oxidase.

Table 6.1: Ethylene production (µl C_2H_4/kg.hr^{-1}) of mesocarp tissue plugs from 'Tommy Atkins' mangoes stored in air or 25% CO_2 in air at 12°C.

Time (minutes)	10	20	33	60		70	90	105
Air	2.82	1.88	1.93	1.57	transferred to 25% CO_2	1.37	0.61	0.92
25% CO_2	0.56	0.98	0.92	0.00	transferred to air	1.92	1.68	1.83

Examining the ethylene production of individual fruits for a few more hours and their ACC levels at the end of the CO_2 treatment (Figure 6.5A) it is possible, again, to determine that the effect of CO_2 on ethylene production occurs by inhibiting the activity of ACC oxidase rather than restricting ACC synthase synthesis and activity as reported by Chaves and Tomas (1984). Breaking down this short period in segments and determining the ACC concentrations and the activity of ACC oxidase, it is apparent that a transient increase in ethylene biosynthesis in the high CO_2 treatments was accompanied by a significant drop in ACC concentration (Figure 6.5B). The threefold increase in ethylene after 30 minutes in the 10% CO_2 atmosphere at 8°C (Figure 6.5A), most probably represents a response of the fruit to an extreme environment that also could involve the activation of ACC oxidase by CO_2 as reported by Dong *et al.* (1992), taking into consideration that CO_2 is required for the activity of ACC oxidase (Kao and Yang, 1982; Smith and John, 1993). Besides, this increase in ethylene is probably sustained by the existing ACC pools, since ACC synthase activity (Table 6.2) did not change significantly under the same conditions as Figure 6.5B, which is in contrast to the report of Chavez-Franco and Kader (1993), who found that pear discs had significantly reduced ACC synthase activity during the first 20 hours of exposure to 20% CO_2 in air at 20°C. The subsequent recovery of ACC concentration in the 10% CO_2 treatment (Figure 6.5A) was most likely a consequence of inhibition of ACC oxidase activity, as ethylene production decreased over the same time period. Alternatively, the recovery of ACC levels could also derive from an upregulation of ACC synthase as a response to either the stress of the high CO_2 environment or to the depletion of ACC concentrations. There was a slight increase in the activity of ACC synthase after 2 hours of exposure to 25% CO_2 (Table 6.2) though these differences were not statistically significant.

Table 6.2: ACC synthase activity (nMoles ACC/g.3hr^{-1}) in mesocarp tissue of 'Tommy Atkins' mangoes stored for 4 hours at 8°C in a flow through containing 25% CO_2 mixed to air.

	Initial	30	60	120	240 minutes
ACC synthase activity	0.169	0.0179	0.0164	0.0192	0.0189
sd	0.0016	0.0029	0.0020	0.0089	0.0061

Slight changes in the activity of ACC synthase could result in significant changes in ACC concentrations, but, most probably, the effects of CO_2 on ACC oxidase are the most influential. This conclusion is supported by the results illustrated in Figure 6.6. These western blots clearly indicate that ACC oxidase was present at the end of the CA storage period. Since there was no ethylene being produced in the 25% CO_2 treatments despite maintenance of ACC at levels not significantly different from the controls, at either temperature (Figure 6.1), this indicates that ACC oxidase activity was reversibly inhibited *in vivo* by CO_2. In fact, the blots indicate that the 25% CO_2 treatment induced *de novo* synthesis of ACC oxidase as observed by Philosophos-Hadas *et al.* (1986) with tobacco leaf discs.

Mizutani *et al.* (1995) showed with *in vitro* assays of ACC oxidase from apple tissue that the enzyme activity increased several times at a specific pH with increasing CO_2 concentrations. These authors also showed that lowering the pH also increased the CO_2 optima for ACC oxidase activity. At pH 7.5, maximum activity was observed at 4% CO_2, while at pH 6.0 the enzyme activity showed no sign of leveling off even at 30% CO_2. *In vitro* activity of ACC oxidase extracted from ripe 'Tommy Atkins' mangoes after a 30-minute exposure to 18% CO_2 in a static system was 1.87 ± 0.28 nl C_2H_4/g. F.W. and, after 2 hours, activity had dropped to 0.87 ± 0.05 nl C_2H_4/g. F.W., which was comparable to the controls incubated under ambient CO_2 concentration (0.72 ± 0.08 nl C_2H_4/g. F.W). The drop in the ACC oxidase activity *in vitro* over time might be due to the oxidation of ascorbate in the assay medium, considering the requirement for ascorbate for enzyme activity *in vitro* (Ververidis and John, 1991) and based on information from the work of

Finlayson and Reid (1994), who determined that ACC oxidase activity was strongly dependent on the presence of even small amounts of ascorbate.

The levels of ACC oxidase activity in the present experiments were comparable to the activity measured by Cua and Lizada (1990) in 'Carabao' mangoes, though almost tenfold lower than the activity of the enzyme from other species like apples (Mizutani *et al.*, 1995) and tomatoes (Smith *et al.*, 1994). Mangoes produced relatively low levels of ethylene compared to other climacteric fruit species in all the experiments conducted, confirming the observations of Burdon *et al.* (1996). What is intriguing, however, is the fact that ACC oxidase *in vivo* is inhibited by CO_2 concentrations of 25% but *in vitro* the behavior of ACC oxidase from mangoes is similar to what has been observed, for example, in apples (Poneleit and Dilley, 1993; Mizutani *et al.*, 1995) and in sunflower roots (Finlayson and Reid, 1994).

A possible mechanism of *in vivo* inhibition of ACC oxidase by CO_2 might be the presence of high levels of ethanol and acetaldehyde induced by the CO_2 atmospheres, as seen in previous chapters. Treatments with ethanol and/or acetaldehyde have been shown to affect ethylene biosynthesis in tomatoes (Saltveit, 1989), peaches and nectarines (Lurie and Pesis, 1992) and mangoes (Burdon *et al.*, 1996). In cultivar 'Keitt' mangoes, Burdon *et al.* (1996) concluded that acetaldehyde either inhibited ACC oxidase activity directly or prevented new protein synthesis. While this conclusion would seem to be supported by the results reported here for the exposure of mangoes to CA at 5, 8 or 12°C, this hypothesis does not hold when examining the period following transfer to air at 20°C, in which increases in ethanol, and consequently acetaldehyde, occurred concurrently with increases in ethylene production rates. Therefore, we still consider that the direct effects of CO_2 concentrations on ACC oxidase activity and, to some extent, on cellular organization are the most important factors in inhibiting ethylene biosynthesis of mangoes under CO_2 atmospheres of 25% and higher.

Conclusions

The effects of CO_2 on ethylene biosynthesis are probably primarily a consequence of reversible inhibition of the activity of ACC oxidase. The mechanism of the inhibition of the *in vivo* activity of ACC oxidase can not be addressed based on the available data. The evidence, however, does not support the suggestion in the literature pointing towards metabolites from the anaerobic pathway, either acetaldehyde or ethanol or both, as inhibitors of ethylene synthesis. In mangoes, the effects of CO_2 on the activity of ACC synthase do not seem to be as evident as in other species.

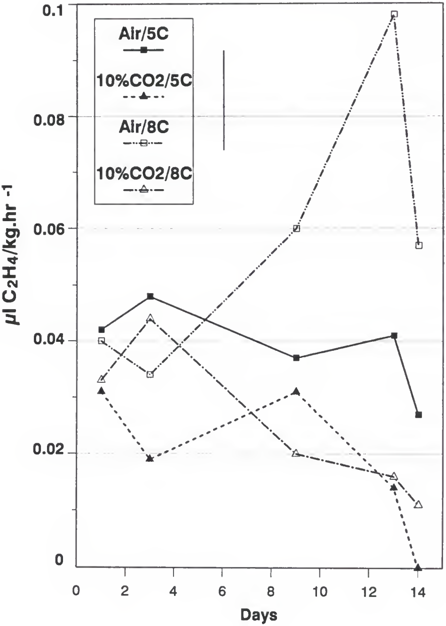

Figure 6.1: Ethylene production of 'Tommy Atkins' mangoes stored for 14 days in CA or air at 5 or 8°C. (Vertical bar = LSD p<.05).

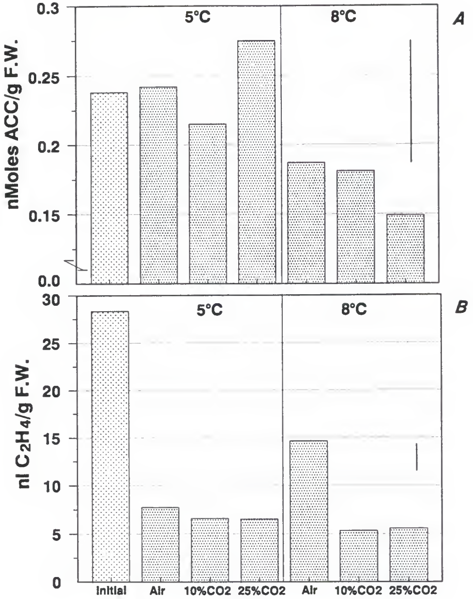

Figure 6.2: ACC concentrations (*A*) and ACC oxidase activity *in vitro* (*B*) in mesocarp tissue of 'Tommy Atkins' mangoes stored for 14 days in CA or air at 5 or 8°C. (Vertical bar = LSD p<.05).

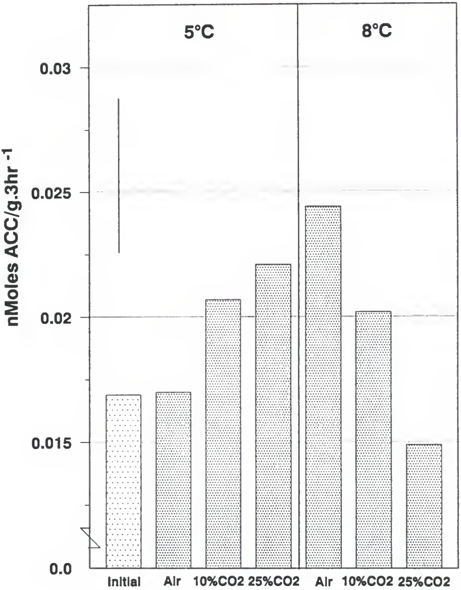

Figure 6.3: ACC synthase activity in mesocarp tissue of 'Tommy Atkins' mangoes stored for 14 days in CA or air at 5 or 8°C. (Vertical bar = LSD p<.05).

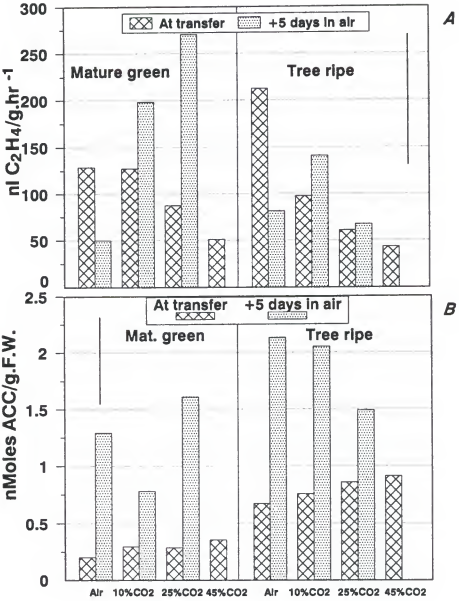

Figure 6.4: ACC oxidase activity *in vivo* (*A*) and ACC concentrations (*B*) in mesocarp tissue of mature green and tree ripe 'Tommy Atkins' mangoes stored for 21 days in CA or air at 12°C plus 5 days in air at 20°C. No measurements were made for fruit from the 45% CO_2 after 5 days in air at 20°C because of CO_2 injury to the tissue. (Vertical bar = LSD $p<.05$).

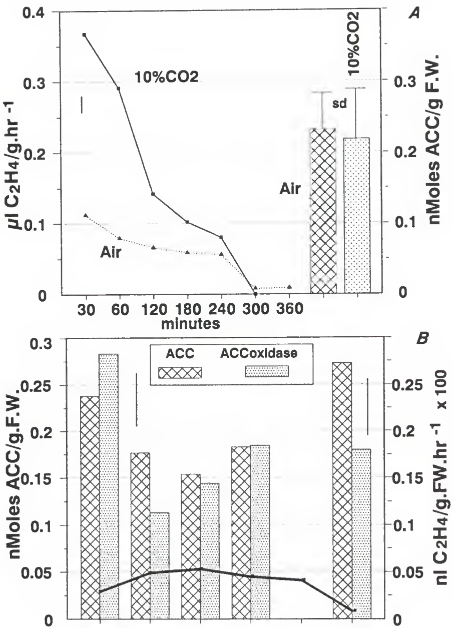

Figure 6.5: Ethylene production (•—•), ACC concentration and ACC oxidase activity *in vitro* in mesocarp tissue of 'Tommy Atkins' mangoes stored at 8°C for 5 hours in 10% CO_2 or air (*A*) or for 4 hours in 25% CO_2 (*B*). (Vertical bars = LSD $p<.05$; left side bar for ACC; right side bar for ACC oxidase; sd = standard deviation).

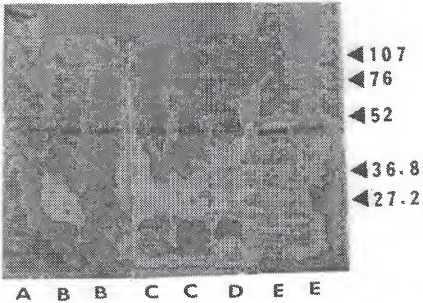

Figure 6.6: Steady state levels of ACC oxidase in mesocarp tissue of 'Tommy Atkins' mangoes stored in 25% CO_2 plus 5% O_2 for 4 hours at 8°C. A = initial; B = 30 minutes; C = 60 minutes; D = 120 minutes and E = 240 minutes. Lines with the same letters are replicates from the same treatment. (Molecular weight, kDa, on the right).

CHAPTER 7
CONTROLLED ATMOSPHERE AND AROMA VOLATILES OF MANGOES

Introduction

Intensified international trade in recent years has promoted mangoes to higher ranks of popularity (Subrahmanyam, 1990; Gourge *et al.*, 1992). Continuous availability on the market has changed the status of mango from that of a specialty fruit with unique flavor (Engel and Tressl, 1983) to a standard item in produce departments. This has been made possible by adoption of postharvest handling practices that enable the movement of larger volumes of fruit (Medlicott *et al.*, 1986; Tucker and Seymour, 1991). The mango industry, however, did not develop a handling procedure for its own use, but rather adapted the practices of the deciduous fruit industry by harvesting the fruit at the preclimacteric ripeness stage, *i.e.*, mature green, and using temperature management and exclusion of ethylene to delay the onset of ripening until after the fruit have reached the destination market. Harvest of mature green mangoes prolongs shelf life (Thomas and Joshi, 1988), but also negatively affects flavor characteristics, as has already been demonstrated for apples (Willaert *et al.*, 1983; Streif and Bangerth, 1988).

Lakshminarayana (1980) determined that mangoes harvested at the mature green stage and then ripened at temperatures above 15°C had better flavor than mangoes held in refrigerated storage below 15°C. There have been a few studies that have attempted to characterize mango flavor components (Engel and Tressl, 1983; MacLeod and Snyder, 1985; Bartley and Schwede, 1987; Koulibaly *et al.*, 1992), but there have not been any systematic determinations, going beyond the use of descriptive terms or hedonic scales (Lakshminarayana, 1980), to evaluate changes in aroma profiles deriving from different storage conditions.

Therefore, by determining aroma volatiles from different mango cultivars that had been stored for up to 3 weeks in various combinations of temperature, ripeness stage and CA storage, the objective of this study was to determine if any of those factors could significantly influence the production of the volatiles present in the mango aroma profile.

Material and Methods

The determinations of aroma volatiles were performed on fruit from 'Haden', 'Keitt', and 'Tommy Atkins' mangoes representing the different ripeness stages, treatments and storage temperatures of the experiments described in the previous chapters. Mesocarp tissue was homogenized at transfer and/or after the ripening period in air at 20°C. A 50% diluted homogenate was prepared by mixing equal amounts of tissue and deionized water. The diluted samples were stored at -20°C until used for volatile determinations.

Volatile components were quantified based on the procedure of Baldwin *et al.* (1991) as described by Malundo (1996) for mangoes using a Perkin-Elmer GC (Foster City, CA), model 8500, equipped with a model HS-6 headspace sampler, a flame ionization detector and a 0.53x3000 mm polar Durowax column (J & W Scientific, Folsom, CA). The concentrations of the volatiles were calculated using regression equations fitted to peak height calibration curves as described in Malundo (1996) except for acetaldehyde and hexanal for which no calibration curves were prepared. These are presented as peak height.

Results and Discussion

A typical GC capillary chromatogram of a diluted mango sample is presented in Figure 7.1. In this specific chromatogram, two components, β-pinene and α-copaene, did not have recognizable peaks in the elution sequence. The 13 compounds that were quantified in the present work probably are, according to Malundo (1996), the most critical for mango flavor, even though Wilson *et al.* (1990) concluded that a typical component for mango flavor can not be determined, considering the complexity of the components in a mango volatile profile. This conclusion was reiterated by Adedeji *et al.* (1992), who further added that the variability in the aroma complex also depends on growing conditions and ripeness stage at harvest.

Engel and Tressl (1983) determined 114 different aroma compounds in two Indian type mango cultivars ('Alphonso' and 'Baladi'), while Idstein and Schreier (1985) detected 152 compounds in 'Alphonso'. Both reports state that about 70 compounds had been identified for the first time in mangoes, but still, Engel and Tressl (1983) considered that this amount of compounds is relatively small compared to the amount of aroma compounds in other fruit species, for example apples, with more than 250 (Willaert *et al.*, 1983).

It has also been questioned by MacLeod and Snyder (1985), when examining aroma profiles of 'Keitt' mangoes, if ethanol and, consequently, acetaldehyde, are true components of mango flavor. It seems, though, that these components are, in fact, compounds of mango aroma considering that acetaldehyde and ethanol were found at the beginning of ripening and before storage (data not shown), contrasting with the statement of MacLeod and Snyder (1985) that only stored or overripe mangoes produce ethanol.

The components found in the present work were predominantly monoterpenes and sesquiterpenes confirming the conclusions of MacLeod and Snyder (1985), who worked with 'Tommy Atkins' and 'Keitt' mangoes. MacLeod and Pieris (1984) also identified these terpenes as the most abundant volatiles in 'Jaffna', 'Willard' and 'Parrott' mangoes, which are polyembryonic cultivars (Lizada, 1993). The component that has been determined to be the most abundant in 'Tommy Atkins', 'Keitt' and 'Kent', the three Florida-type mangoes examined by Malundo (1996), is 3-carene, a monoterpene hydrocarbon (Adedeji *et al.*, 1992), which has also been verified in our experiments, confirming the observation of MacLeod and Snyder (1985).

In the experiments with 'Haden' and 'Tommy Atkins' mangoes in which the response to the reduction of O_2 concentrations was tested (Chapter 4), there was no treatment effect on 3-carene elution from mesocarp tissue when sampled on the day of transfer to air at 20°C (Table 7.1). 'Haden' had higher production of 3-carene as well as limonene and p-cymene than 'Tommy Atkins'. This difference could be a consequence of the higher storage temperature used for 'Haden' fruit according to the observations of Bandyopadhyay and Gholap (1973) and Lakshminarayana (1980), but α-pinene, also a terpenoid hydrocarbon (Idstein and Schreier, 1985) and, therefore, supposedly deriving from the same general pathway, was significantly higher in 'Tommy Atkins', suggesting that cultivar differences are also major factors with regard to the differences in aroma compounds.

Table 7.1: Aroma compounds in mesocarp tissue of 'Haden' and 'Tommy Atkins' mangoes at transfer from storage for 2 weeks at 15°C or 3 weeks at 12°C, respectively.

	α-pinene (ppm)	3-carene (ppm)	limonene (ppm)	p-cymene (ppm)	acetaldehyde (peak height)	hexanal (peak height)
'Haden'						
Air	0.318	34.32	0.591	0.0903	1317.59	1020.78
2% O_2	0.160	18.89	0.310	0.0458	3511.80	1271.32
5% O_2	0.085	9.51	0.151	0.0374	730.58	1359.09
25% CO_2	0.092	11.49	0.196	0.0974	743.99	1191.87
'Tommy Atkins'						
Air	1.493	9.80	0.154	0.0188	1757.30	337.71
2% O_2	1.648	8.88	0.180	0.0154	16385.37	390.54
5% O_2	0.970	6.31	0.111	0.0041	8111.74	218.80
25% CO_2	1.187	8.86	0.152	0.0150	1359.72	129.39
LSD $p<.05$/[1]	0.331	10.02	0.139	0.0338	2525.90	482.30

/[1] LSD for the differences between cultivars.

Cultivar differences were also observed in relation to acetaldehyde and hexanal peak height, which is indicative of sample concentration. In addition to this difference between the two cultivars, there was also a significant difference in acetaldehyde peak height among the treatments. Both 'Haden' mangoes stored for 14 days and 'Tommy Atkins' mangoes stored for 21 days in the 2% O_2 atmosphere had the highest acetaldehyde peaks for the cultivar, though only with 'Tommy Atkins' was this height significantly different from the other treatments. Fruit from the 2% O_2 treatment in both cultivars also had the highest ethanol production rates, as discussed in Chapter 4. Acetaldehyde peak height in the 5% O_2 treatment for 'Tommy Atkins' was also significantly higher than the control and 25% CO_2 treatments, indicating that reduction of the O_2 concentration in the storage atmosphere for mangoes affects anaerobic metabolism not only by stimulating

ADH, but also PDC, as observed by Ke *et al.* (1993) in strawberries and Ke *et al.* (1995) in avocados.

There was no temperature effect on 3-carene, α-pinene and limonene in the samples of 'Tommy Atkins' mangoes analyzed after 2 days in air at 20°C (Table 7.2), when comparing storage of tree ripe fruit at 8°C to storage at 12°C, though, p-cymene was significantly lower in the mangoes stored at 8°C. There was a tendency to lower concentrations of these terpenes in the 25% CO_2 treatment at both temperatures and in both ripeness stages. Acetaldehyde levels were significantly higher in the tree ripe mangoes from the 25% CO_2 treatment.

Table 7.2: Aroma compounds in mesocarp tissue of mature green and tree ripe 'Tommy Atkins' mangoes stored for 3 weeks at 8 or 12°C.

	α-pinene (ppm)	3-carene (ppm)	limonene (ppm)	p-cymene (ppm)	acetaldehyde (peak height)	hexanal (peak height)
Mature green at 12°C						
Air	2.564	18.58	0.305	0.030	1322.4	360.63
10% CO_2	2.350	12.67	0.280	0.033	1309.9	260.55
25% CO_2	0.756	5.35	0.125	0.000	1314.2	213.19
LSD ($p<.05$) /[1]	3.120	25.30	0.479	0.063	NS	109.49
Tree ripe at 12°C						
Air	7.925	63.29	1.319	0.094	1498.4	165.01
10% CO_2	6.671	58.44	1.044	0.148	2178.4	157.50
25% CO_2	5.731	52.63	0.863	0.080	11802.5	119.50
Tree ripe at 8°C						
Air	7.214	58.65	0.898	0.039	1459.2	91.80
10% CO_2	5.857	53.52	1.029	0.036	1576.9	28.50
25% CO_2	2.065	14.76	0.338	0.000	66886.9	48.18
LSD ($p<.05$)/[2]	NS	NS	NS	0.068	28191.7	84.34

/[1] LSD for the comparison between mature green and tree ripe mangoes stored at 12°C.

/[2] LSD for the comparison between tree ripe mangoes stored at 8 or 12°C.

In the experiment with 'Tommy Atkins', there was a significant difference in production of all the terpenes and also hexanal between mature green and tree ripe mangoes stored at 12°C. Acetaldehyde levels were not significantly different because of high variation between replicates. Mature green 'Tommy Atkins' mangoes had significantly lower levels of terpenes but higher concentrations of hexanal than the tree ripe fruit stored at the same temperature.

In the samples of 'Keitt' mangoes analyzed after 3 days in air at 20°C, only the fruit stored in the 25% CO_2 atmosphere at 5°C had significantly higher levels of 3-carene, α-pinene and limonene, while p-cymene was completely absent in the mangoes from this treatment (Table 7.3). Acetaldehyde was significantly higher in the 25% CO_2 treatment, while hexanal levels were significantly lower in this treatment. The 10% CO_2 treatment at 5°C was also significantly lower than the two controls.

Table 7.3: Aroma compounds in mesocarp tissue of 'Keitt' mangoes after 3 days in air at 20°C following a 21-day storage in CA or air at 5 or 8°C.

	α-pinene (ppm)	3-carene (ppm)	limonene (ppm)	p-cymene (ppm)	acetaldehyde (peak height)	hexanal (peak height)
Air-5°C	0.0327	3.153	0.0527	0.0046	1270.45	293.02
Air-8°C	0.0410	3.984	0.0672	0.0098	1464.87	303.50
10% CO_2-5°C	0.0260	3.578	0.0583	0.0061	808.40	161.02
25% CO_2-5°C	0.1725	6.569	0.0959	0.0000	18270.60	64.49
LSD $p<.05$	0.0626	1.194	0.0188	0.0020	3224.89	82.92

'Keitt' mangoes from the 25% CO_2 treatment at 5°C showed CO_2 injury symptoms. There were visual symptoms on the epidermis as well as internal symptoms, indicated by the significantly higher ACC concentrations in comparison to control fruit stored at 5°C, which had an indication of chilling, *i.e.*, higher electrolyte leakage and ethylene production, even though no visual symptoms were detected (Figure 7.2). This treatment already had the same amount of ion efflux at the time of transfer from storage at 5°C as after 3 days at 20°C, as discussed in Chapter 5. Except for p-cymene, all the compounds of the 5°C control fruit had volatile levels similar to the control fruit at 8°C in which ripening proceeded unhindered as discussed in Chapter 5.

The 25% CO_2 treatment seemed to have different effects on the biosynthesis of aroma compounds depending on the ripeness stage of the fruit. At the early stages of ripening, high CO_2 storage atmosphere could have a greater effect on the synthesis of volatile compounds, which could lead to poor flavor in the mature green fruit after transfer to air, as determined with 'Tommy Atkins' (Table 7.2). With tree ripe fruit, the same atmosphere, in association with the 5°C storage temperature, might prevent the breakdown and also the synthesis of a few other compounds, like p-cymene (Table 7.3), which could render an uncharacteristic flavor to the mangoes stored under these conditions.

Lipid components and their breakdown to fatty acids have been considered to be the main precursors of volatiles in mangoes and in fruits in general (Gholap and Bandyopadhyay, 1980; Selvaraj *et al.*, 1989; Koulibaly *et al.*, 1992). Beyond this vague statement regarding aroma compound biosynthesis, there is still much left to investigate in terms of specific pathways leading to important compounds for the aroma of mangoes. How each one is regulated and responds to CA and more severe reduction in the storage temperature needs further investigation dependent on the elucidation of the pathways.

Conclusions

Among the aroma compounds analyzed, p-cymene biosynthesis seemed to be most sensitive to storage in 25% CO_2. This sensitivity appeared to be enhanced in mature green fruit and by storage at 8°C or below. Tree ripe mangoes stored in an atmosphere of 25% CO_2 produced significantly higher levels of acetaldehyde. The 25% CO_2 treatment at 5°C had the most pronounced effect on the aroma profile of 'Keitt' mangoes, which suggests that this treatment causes damage that severely impairs ripening after transfer to air.

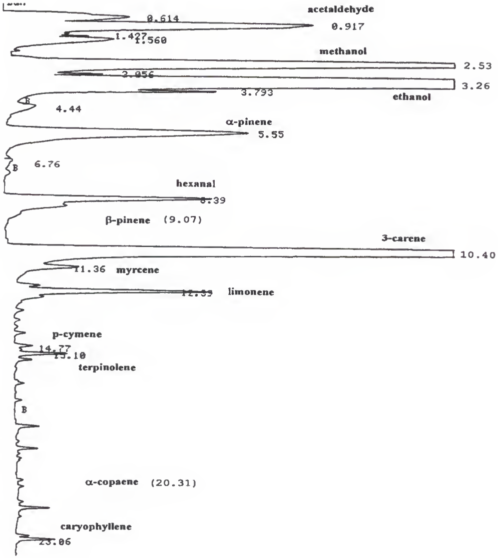

Figure 7.1: Representative capillary elution sequence from headspace aroma sample of 'Tommy Atkins' mango. β-pinene and α-copaene were not detected in this specific sample and their retention times are indicated in parenthesis.

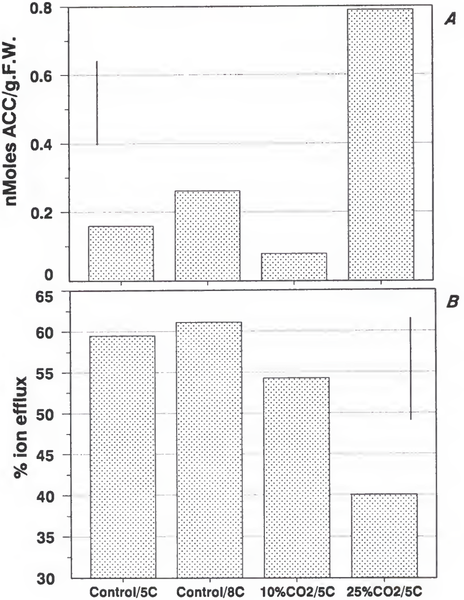

Figure 7.2: ACC concentrations (*A*) and electrolyte leakage (*B*) of mesocarp tissue of 'Keitt' mangoes stored for 3 days in air at 20°C following storage in 10 or 25% CO_2 at 5°C or air at 5 or 8°C for 21 days. (Vertical bar = LSD p<.05).

CHAPTER 8
CONCLUSIONS

The first objective in this study was to examine the applicability of long term CA storage of mangoes with elevated CO_2 concentrations. Based on the relatively short storage life of mangoes and considering that current harvesting and handling practices do not result in good fruit quality reaching the market, a second objective was to investigate the effectiveness of reducing the storage temperature together with CA to prolong the storage life of mangoes, which could make a tree ripe program for mangoes more feasible.

From the experiments in which CO_2 concentrations of 35% and above were tested it was concluded that these concentrations for long term storage are beyond the tolerance limit of mangoes. These high CO_2 atmospheres caused severe damage and the fruit did not resume proper ripening processes after transfer to air at 20°C. Respiration rates and ethanol production rates during storage were significantly higher than that of control fruit stored in air at the same temperature. Epidermal color was abnormal, while the color of the mesocarp was pale. Ethylene production was drastically suppressed in CO_2 atmospheres above 25%. The suppression of ethylene biosynthesis under CO_2 concentrations of 25% and above are primarily a consequence of a reversible inhibition of the activity of ACC oxidase. This inhibition, however, most likely does not derive from metabolites of the anaerobic pathway as suggested in the literature. The effects of CO_2 concentrations on the activity of ACC synthase in mangoes does not seem to be as evident as in other species.

From these experiments it became apparent that O_2 concentrations had little additional effect on the inhibition of ripening processes during storage compared to CO_2. In order to evaluate more precisely this information, the effects of O_2 concentrations on mango storage were tested. The reduction of O_2 concentrations in the storage atmosphere significantly enhances ethanol production. The 2% O_2 storage treatment had a residual effect on respiratory activity and ethylene production after transfer to air. Epidermal color development of the 2% O_2 and the 25% CO_2 treatments was slower after transfer to air at 20°C, which is probably due to the inhibition of ethylene action by low O_2 and high CO_2. Quality parameters were not significantly affected by the reduced O_2 atmospheres.

Storage of tree ripe mangoes in CA at 12°C is not to be a viable alternative to air storage because already initiated ripening processes are not considerably hindered. On the other hand, the reduction of the storage temperature from 12 to 8°C has a beneficial effect on mango quality. Tree ripe mangoes stored in 10 or 25% CO_2 plus 5% O_2 at 8°C had the best quality after 21 days plus 3 or 5 days in air at 20°C compared to air storage at 8°C and air and CA storage at 12°C.

An even further reduction of the storage temperature to 5°C caused some damage to the fruit. The control fruit in air at 5°C had indications of chilling injury, *i.e.*, higher electrolyte leakage and ethylene production, though no visual symptoms were observed after 3 days in air at 20°C. The fruit from 25% CO_2 storage showed visual symptoms of CO_2 injury at the time of transfer from CA, but these symptoms, which were confined to the epidermis, did not worsen after 3 days in air. A storage atmosphere of 5% O_2 plus 10% CO_2 at 5°C significantly inhibited ripening compared to the air control without developing symptoms of either chilling injury or CO_2 injury.

Storage of mangoes in 25% CO_2 atmospheres has a significant effect on the elution of aroma volatiles. Among several monoterpene hydrocarbons, considered the most critical for flavor of the New World mango cultivars, p-cymene biosynthesis was the most sensitive to the 25% CO_2 atmosphere. Besides completely inhibiting the synthesis of p-cymene, this treatment, along with the 2% O_2 treatment, significantly enhanced acetaldehyde production of tree ripe mangoes. The 10% CO_2 atmosphere does not seem to critically affect mango flavor at any of the storage temperatures.

From the results of the present experiments, the recommendation for mango long term storage, which may allow marine transport to supply distant markets, is to harvest the mangoes at the tree ripe stage and hold the fruit in an atmosphere containing at least 10% CO_2 plus 5% O_2 at a storage temperature of 8°C.

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BIOGRAPHICAL SKETCH

The author was born on January 11, 1954, in Sao Sebastiao do Cai, a small town in Rio Grande do Sul, the southernmost state of Brazil. He attended the Federal University of Rio Grande do Sul (UFRGS), where he graduated with a Bachelor of Science degree in Agronomy in 1977. After working for almost three years in the private sector, he joined the Post-Graduate Program in Agronomy at UFRGS for a Master of Science degree, which was awarded in 1982. Before coming to the University of Florida for doctoral studies in 1992, he held a seven-year position of research specialist in conventional and controlled atmosphere storage of apples and pears at a research unit of the Santa Catarina state secretary of agriculture, which was followed by two years as an assistant professor in the Horticultural Sciences Department at UFRGS. After receiving his Ph.D., the author will return to the position at UFRGS.

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

J K Brecht

Jeffrey K. Brecht, Chair
Professor of Horticultural Science

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

Donald J. Huber
Professor of Horticultural Science

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

Steven A. Sargent
Associate Professor of Horticultural Science

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

Jerry A. Bartz

Jerry A. Bartz
Associate Professor of Plant Pathology

This dissertation was submitted to the Graduate Faculty of the College of Agriculture and to the Graduate School and was accepted as partial fulfillment of the requirements for the degree of Doctor of Philosophy.

December, 1996

Jack L. Fry

Dean, College of Agriculture

Dean, Graduate School